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Morphological Identification of Veterinary Important Mosquitoes in the Selected Study Districts of Afar Region of Eastern Ethiopia

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Abstract: Mosquito survey study was conducted from March to August 2021 to identify veterinary important mosquitoes morphologically in Amibara, Gewane and Asayita districts situated in Afar region of Eastern Ethiopia. Trapping sites were selected based on the ecological significance for introduction and maintenance (mosquito breeding sites) and located within the range of geographical area of 9°11' 41.0712" N - 11°19' 22.4796" N, 40°6' 38.448"- 41°15' 54.0612" E and 358-736 m.a.s.l, altitudinal range. From the total 689 captured mosquitoes after examining their morphological features 369 mosquitoes were identified up to genera level: 204 (29.6%) *Aedes*, 140 (20.3%) *Culex* and 25 (3.6%) *Anopheles*. The remaining 320 mosquitoes identified further at species level and the existence of 11 mosquito species were confirmed in the study area in the following decreasing order of their count: *Culex pipines* and *Mansonia uniformis* which have equal count each 135 (19.6%), *C. antennatus* 28 (4.1%), *Anopheles arabiensis* 10 (1.5%), *Aedes aegypti*, *A. vexans, A. mcintoshi, C. theileri, An. plumbes,* which also have equal count each 2 (0.3%) *A. cumminsii* and *C. perexiguus* with the least count 1(0.1%). There was significant statistical difference in the catch of mosquitoes between districts and between species (P<0.05). Nowadays mosquito-borne diseases increased due to globalization and the finding of this study might contribute in the selection of effective and sustainable methods for controlling these vectors even though it lack behavioral information of these vectors/mosquitoes which need farther studies.

Key words: Vector-Borne · Mosquito · *Aedes* · *Culex* · *Mansonia* · *Anopheles*

INTRODUCTION

Mosquito is a small winged insect belongs to the order Diptera and the family Culicidae which is divided into 3 subfamilies (Anophelinae, Culicinae and Toxorhynchitinae) that consists of 3,490 currently recognized species grouped in 41 genera and among these the greatest species diversity occurs in tropical forest. From these sub families Anophelinae and the Culicinae are blood feeders but the third subfamily, the Toxorhynchitinae, do not feed on blood [1, 2].

Adult mosquitoes are small, slender insects of about 3 to 6 mm in length (0.15-0.4 inches). Males have feathery antennae; those with short antennal hairs are females. Mosquitoes use a complex set of cues, long range, mid-range and close range to find a host [3]. They generally feed on plant nectar, but females of most species are parasitic and must consume blood to gain nutrients and proteins needed to produce their eggs and depend on diversity of hosts, ranging from mammals and birds to reptiles, amphibians an even fish [4].

Female mosquito lay between 30 to 300 brown to blackish eggs at one time. *Anopheles* eggs are boat or oval shape and float on the water. *Culex* and some *Mansonia* eggs are also laid on the water, but are deposited in the form of rafts. Eggs of these three species cannot survive desiccation. *Aedes* lay their eggs in damp places just beyond the water line. Some *Aedes* prefer tree holes, clay pots and other containers. *Aedes* eggs can with stand weeks or years of desiccation and can survive cold weather. Hatching is triggered by alternate cycles of flooding and drought and do not hatch all the eggs at the same time. These mosquitoes tend to be timed release pests and never go away without good control measures. Most mosquito larvae must come to the surface to breath. At this time they are most vulnerable and this is the

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reason that mosquito control work focuses on the larval stage. *Anopheles* larvae lie parallel to the water surface and breathe through the holes in their sides called spiracles. Mosquito's importance as pests is insignificant compared with their role as vectors particularly of human diseases such as malaria. *Anopheline* mosquitoes also transmit malaria parasites to other animals, for example *Plasmodium knowlesi* and *P. cynomolgito* to monkeys and *P. bergheito* to rodents. *Culicine* mosquitoes transmit malaria parasites such as *P. gallinaceum* to wild and domesticated birds. *Anopheles gambiae* is one of the best known, because of its predominant role in the transmission of the most dangerous *Plasmodium falciparum* [5]. Some species of *Anopheles* can also serve as the vectors for canine heart worm *Dirofilaria immitis*, the *Filariidae wuchereria bancrofti* and *Brugia malayi* and viruses [6].

Culicinae larvae hang from the surface at angle and breathe through a siphon tube. Many species spend 5-7 days in the larval stage. Mosquitoes can develop anywhere, where there is standing water. The range of habitats is wide. Fresh water, salt water, brackish water, ground pools, wells, cesspools, marshes, containers, tires, tree holes and aquatic vegetation are all areas where mosquitoes can develop [7].

Culex pipiens is primarily interested in birds and poultry, but also attacks humans and other mammals was a vector of St. Louis encephalitis in U.S. epidemics. It is also a vector of the canine heart worm and filariasis in humans. A related species of *Culex* species *Culex tasalis* is a principal vector of western equine encephalitis. Eastern equine encephalitis spread to man and horses by *Aedes* species [8].

Mechanical transmission of a number of poxviruses by biting arthropods is well documented and includes myxoma virus where *Ae. aegypti* has been identified as an important vector [9]. Mosquitoes have also been shown to mechanically transmit *Shope fibroma* virus and fowl pox virus [10, 11]. In the 1959 Kenyan outbreak of lamp skin disease, there were reports of high infestation of *Aedes natronius* and *Culex mirificus* [12].

In addition mosquito-borne arboviral diseases are becoming big health challenge of human and animal worldwide [13]. For example Rift Valley Fever (RVF) is adapted to wide range of vectors, predominantly mosquitoes [14]. The virus has been isolated from more than 53 mosquito species in 8 genera in regions where epizootics occurred [15]. It causes almost 100% mortality rates among young animals and high abortion rates among livestock [16]. The mosquito vectors transmitting RVF can be classified into two major groups, namely primary and secondary vectors [14]. Floodwater mosquitoes of genus *Aedes* have been considered the primary maintenance host and source of RVFV that initiate disease outbreaks [14, 17, 18]. The genera *Culex*, *Anopheles*, *Eretmopodites* and *Mansonia* constitute the secondary vectors which take over flooded grounds for breeding, contribute to the amplification of the virus due to their ubiquitous biting patterns, consequently resulting in outbreaks [14, 19]. *Aedes ochraceus* and *Aedes mcintoshi* is the most important maintenance vector of RVFV in East and Southern Africa. Similarly, *Aedes vexans* were found to be responsible for a large outbreak in West Africa and the likely maintenance vector in Saudi Arabia during the emergence of RVFV in 2000 [20]. Further more researchers in the field pointed out the importance of mosquitoes in the transmission of various diseases to animals and to human: for example the overall mosquitoes importance in the transmission of pathogens that causes of arboviral diseases (avian pox, bovine ephemeral fever, dengue fever, Rift Valley fever, West Nile fever), bacterial diseases (Anthrax, Tularemia), helminthic diseases (mosquito-borne filariasis), protozoans (Avian malaria, Human malaria) were indicated in the work of Azari-Hamidian S. and his colleagues[21]. Besides their role in the transmission of diseases Garros C. and his coworkers had also indicated that mosquito bites may cause stress and pain resulting in the reduction of livestock fitness [22].

Identification of mosquito species is one important step to control mosquito borne-diseases. It allows acquiring biological information such as breeding sites, biting and resting habits that differ among mosquito species [23]. Although they have similar morphology, mosquitoes have considerable differences in ecological, epidemiological significance and physiological features, including food preferences [24]. This helps to improve efforts to understand the spatial epidemiology of arboviruses and to predict how these could change in the future and could be used for early warning detection and implementation of control measures are essential.

In Ethiopia entomological study, regarding veterinary important mosquito has not been investigated. Therefore, this study with the following objective aimed to fill this gap.

Objective: The objective of this study is to identify veterinary important mosquitoes morphologically in the selected parts of Afar regional state at genus and species level.

MATERIALS AND METHODS

Study Area: The study was undertaken in seven selected sites in Zone 3 at Amibara district in Serkamo and Shelako PAs at Gewane district in Galela dura and Entaye hadota PAs in Asayita Zone at Berga dura and Sahale PAs of Afar regional state where the sites were selected based on the ecological significance for introduction and maintenance (mosquito breeding sites). These sites located within the range of geographical area of 9° 11' 41.0712" N - 11°19' 22.4796" N, 40°6' 38.448"- 41°15' 54.0612" E and 358-736 m.a.s.l, altitudinal range.

Karma is the main rainy season in Afar which starts as heavy and stormy rains during early to mid-June, peaking during the month of July and continuing with lighter and lighter showers during the month of August and very limited and declining during early to mid-September. The current survey study was conducted from March to August 2021 in the area after the heavy rain which causes flooding in most parts of the study areas. The main perennial river flowing in the area is Awash but in the current study small rivers like Galela dura, Berga dubura and Sahale were used to position the mosquitoes trap.

People are predominantly involved in small-scale subsistence agriculture production and mainly on livestock husbandry and rear different animals, predominantly cattle, sheep, goat and camels. According to CSA, 2020/21[25] reports the region has 1,959,185 cattle, 4,476,485 sheep and 8,843,082 goats.

Study Population: The study animals for this particular study were Mosquitoes that were collected from the studied three districts.

Study Type: The study type was convenience sampling method to collect Mosquitoes in different selected trap sites of Amibara, Gewane and Asayita districts.

Mosquito Collection and Identification

Mosquitoes Were Collected by Using Two Different Types of Traps: CDC light traps and Modified Onderstepoort UV light traps. CDC light trap baited with sugar-yeast solution where by its fermentation reaction release carbon dioxide which is an important mosquito attractant.

The traps were set in proximity to potential mosquito breeding and feeding sites include indoor and outdoor (near water bodies near animal pen and on field of where there are human and livestock population). The trap placed at 18:00 PM and collected at 6:00 -7:00 AM in succeeding day. The next morning the traps were collected and using collection cup and put in deep freeze (-20°C) for 15 minutes to kill mosquitoes, then each specimen of mosquito inserted in to cryovial tubes which has silica gel to absorb moisture and cotton to protect from breaking. Sorting and identification into genus and species level were made by using dichotomous keys of Walter Reed Bio Systemic Unit (WRBU) Potter[26] and Edwards [27] under the aid of a stereo light microscope.

Data Management and Analysis: All data obtained from the study were entered and managed into Microsoft Excel worksheet and the analysis was made using Chi-square (x^2) test in SPSS version 20.

RESULTS

A total of 689 adult mosquitoes were collected from the three districts of Afar region of Eastern Ethiopia and the highest catch was from Amibara district 453(65.7%) followed by Gewane district 185 (26.9%) and the least catch was that of Asayita district 51 (7.4 %) (Table 1). After examining their morphological features 369 mosquitoes from the total 689 catch were identified up to the following genera level: 204 (29.6%) *Aedes*, 140 (20.3%) *Culex* and 25 (3.6%) *Anopheles*. The remaining 320 mosquitoes identified further at species level and the existence of 11 mosquito species were confirmed in the study area in the following decreasing order of their count: *Culex pipines* and *Mansonia uniformis* which have equal count each 135 (19.6%), *C. antennatus* 28 (4.1%), *Anopheles arabiensis* 10 (1.5%), *Aedes aegypti*, *A. vexans, A. mcintoshi, C. theileri, An. plumbes,* which also have equal count each 2 (0.3%) *A. cumminsii* and *C. perexiguus* with the least count 1(0.1%) (Table 1). There was significant statistical difference in the catch of mosquitoes between districts and between species too ($P<0.05$).

Table 1: Mosquito species identified in three districts at Afar region of Eastern Ethiopia

Mosquito species		District Amibara	Gewane	Asyita	Total
Aedes	Count	126	45	33	204
	% within Mosquito Species	61.80	22.10	16.20	100.00
	% within District	27.80	24.30	64.70	29.60
Culex	Count	107	15	18	140
	% within Mosquito Species	76.40	10.70	12.90	100.00
	% within District	23.60	8.10	35.30	20.30
Anopheles	Count	25	0	0	25
	% within Mosquito Species	100.00	0.00	0.00	100.00
	% within District	5.50	0.00	0.00	3.60
M.uniformis	Count	47	88	0	135
	% within Mosquito Species	34.80	65.20	0.00	100.00
	% within District	10.40	47.60	0.00	19.60
A.aegypti	Count	2	0	0	2
	% within Mosquito Species	100.00	0.00	0.00	100.00
	% within District	0.40	0.00	0.00	0.30
A.cumminsii	Count	1	0	0	1
	% within Mosquito Species	100.00	0.00	0.00	100.00
	% within District	0.20	0.00	0.00	0.10
A.vexans	Count	1	1	0	2
	% within Mosquito Species	50.00	50.00	0.00	100.00
	% within District	0.20	0.50	0.00	0.30
A. mcintoshi	Count	2	0	0	2
	% within Mosquito Species	100.00	0.00	0.00	100.00
	% within District	0.40	0.00	0.00	0.30
Cx. pipines	Count	115	20	0	135
	% within Mosquito Species	85.20	14.80	0.00	100.00
	% within District	25.40	10.80	0.00	19.60
Cx.antennatus	Count	17	11	0	28
	% within Mosquito Species	60.70	39.30	0.00	100.00
	% within District	3.80	5.90	0.00	4.10
Cx. theileri	Count	1	1	0	2
	% within Mosquito Species	50.00	50.00	0.00	100.00
	% within District	0.20	0.50	0.00	0.30
Cx.perexiguus	Count	1	0	0	1
	% within Mosquito Species	100.00	0.00	0.00	100.00
	% within District	0.20	0.00	0.00	0.10
An.arabiensis	Count	8	2	0	10
	% within Mosquito Species	80.00	20.00	0.00	100.00
	% within District	1.80	1.10	0.00	1.50
An.plumbes	Count	0	2	0	2
	% within Mosquito Species	0.00	100.00	0.00	100.00
	% within District	0.00	1.10	0.00	0.30
Total Mosquito	Count	453	185	51	689
	% within Mosquito Species	65.70	26.90	7.40	100.00
	% within District	100.00	100.00	100.00	100.00

(X^2 =201.510 Df =26 and P-Value < 0.05)

DISCUSSION

In comparison with other studies like with the study of Kinyatta and his colleagues [28] who conducted the study in Kenya; neighboring country of Ethiopia and collected 1632 mosquitoes which they identified in to five genera (*Culex*, *Aedes*, *Anopheles*, *Mansonia* and *Ficalbia*), the collected mosquitoes number was found to be lesser and the *Ficalbia* mosquito genera was absent in the current study mosquitoes collection. This might be associated with the limitation of time and spatial coverage of the study areas. But in agreement with the study of Ridha M [29] from the collected mosquito species highest catch were recorded in the mosquito species of *Mansonia* and *Culex*. In spite of its very low abundance in the current mosquito species collections, *A. mcintoshi* have been indicated in Kenya being the predominant mosquito and the most known RVF vector [14, 18].

Cx. pipines, Cx. theileri and *Cx. perexiguus* are among the captured species in the current studies whereby their role to transmit many species of avian plasmodium were mentioned in the studies of researchers in different countries [30, 31]. *Cx. Pipines* in Africa and *Cx. perexiguus* in Asia and Europe have been also reported being the principal vectors of West Nile Virus (WNV) [32, 33]. The role of *Cx. Pipines* and *Cx. Perexiguus* in the transmission of RVFV among humans and animals have been mentioned also by Turell and his colleagues [34].

A. aegypti which is captured in this study is mentioned with other vectors being a vector of Lumpy skin disease (LSD) [35].

Flood water *Aedes* species; *A. mcintoshi*, sighted in the work of Rosemary and his co-workers [36] being the primary vector of RVF and in the neighboring country of Kenya the 2006/2007 outbreak of RVF and its transmission was also associated with this mosquito species.

The Epizootics of RVF in East and South Africa and its association with *A. mcintoshi*, *A. cummonsi* and *A.vexans* mosquito vectors have been explained in the study of Meegan and Bailey [37] these mosquito species were also caught in the current study although the virus isolation work was not conducted.

Among other mosquito genera the importance of Mansonia in the transmission of nematode have been indicated in the work of Anderson [38] and in the current study from the captured mosquito species *M.uniformis* has got the highest count like *Cx. Pipines.*

CONCLUSION AND RECOMMENDATION

The current study shows that the study area harbor 9 Culicinae species namely: *A. mcintoshi*, *A. cummonsi* and *A.vexans*, *A. aegypti*, *Cx. pipines*, *Cx. theileri*, *Cx. Perexiguus, C. antennatus*, *M.uniformis* and 2 Anophelinae species ; *Anophele*s *arabiensis* and *An. Plumbes* because of the diseases they transmit these mosquitoes are the greatest enemies of humans and their livestock.

Nowadays mosquito-borne diseases has also increased due to globalization and the finding of this study might contribute in the selection of the effective and sustainable methods for controlling these vectors. Since this study lack other important behavioral information of these vectors/mosquitoes ; such as their biting rate, host preference, dispersal and the effects of these behaviors on vector mortality and population growth; which are important in understanding the vector-borne disease (VBD) dynamics; which in turn important in the application of the control method. Therefore this study needs to be further strengthened by scaling up its scope to fill its gap.

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