

**A REPORT ON ENTOMOLOGICAL SURVEY OF ANOPHELES
COMPOSITION IN BARDAGORIYA RURAL MUNICIPALITY
KAILALI DISTRICT, NEPAL**

(2078/01/24- 2078/01/30)



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Submitted to:

Sudurpashchim Pradesh

Ministry of Social Development

Provincial Health Directorate

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ACKNOWLEDGMENT

We would like to express our immense gratitude to Health Directorate, Rajpur, Doti for providing us such a golden opportunity to conduct entomological surveillance in Kailali, Sudurpashchim Pradesh. Our team is also thankful to all the committee members, public health officers, vector inspectors, village leaders, village malaria workers, and volunteers for their continuous assistance and dedication throughout the completion of this work.

ABSTRACT

Malaria is one of the leading health problem in the Sudurpashchim Pradesh of Nepal. Entomological surveillance is an essential component of malaria vector control programmes, operational activities and research. Despite of several epidemiological malaria interventions implemented to achieve the target of Malaria Elimination Program by 2025, there is still paucity of information about entomological surveillance. The survey was carried out from 2078/01/24 to 2078/01/30 in Bardagoriya Rural Municipality of Kailali District. This study aimed to expand the current knowledge on the distribution and species composition of *Anopheles* in the study area. *Anopheles* mosquitoes were collected by the Centers for Disease Control and Prevention (CDC) Light Traps Catches and by using aspirators with the support of torch light from ten sampling household of each sampling sites. *Anopheles* mosquitoes were identified morphologically and taxonomically up to species level using standard keys. Abundance of *Anopheles* residing in both indoor and outdoor mixed dwellings (human houses and cattle sheds) during morning and night time was calculated. Out of 118 female *Anopheles* collected specimens, four species were identified during this study. The most abundant species was *An. annularis* (42.37%) whereas the least abundant species was *An. splendidus* (0.85%) during morning indoor collection. During night collection, among 86 female *Anopheles* specimens, four *Anopheles* species were identified representing *An. annularis* (36.67%), *An. subpictus* (30%), *An. culicifacies* (28.89%) and *An. splendidus* (4.44%). Similarly, the study has shown the high proportion of freshly fed female *Anopheles* (43.22%) during morning indoor hand collection in this sampling site. Moreover, the presence of high proportion of the potential malaria vectors such as *An. annularis*, *An. culicifacies*, *An. splendidus* as well as *An. subpictus* has indicated a threat of malaria transmission within study area as well as its surroundings. Most of the *Anopheles* mosquitoes were collected from cattle sheds mixed with human dwellings in villages, showing the variation in mode of resting and feeding behavior. Hence, human and vector contact should be minimized throughout the year together with long-lasting insecticide-treated nets and insecticide residual sprays along with other appropriate vector control strategies to reduce the malaria transmission in study area.

1. INTRODUCTION

1.1. Background

Malaria is a complex disease caused by protozoan parasites (Genus: *Plasmodium*) and transmitted by blood-feeding infectious *Anopheles* mosquitoes [1]. Mosquitoes, like any arthropods, are bilaterally symmetrical belonging to the family Culicidae. These are delicate, slender flies; legs are two or three times as long as body, antennae filiform. Body is strongly humped; both head and abdomen drooping downwards. Larvae and pupae are aquatic. A total of 3,585 extant species of Culicidae are currently formally recognised. (2021 March 1, Mosquito Taxonomic Inventory). Over 3,500 mosquitoes have been recorded worldwide [2] however, about 537 species are *Anopheles* [3] and only 70–80 is known to transmit human malaria worldwide [1]. Of these, 41 are considered to be the dominant vector species and capable of transmitting malaria by large [4].

Search of mosquitoes in Nepal was started in connection with "Malaria Eradication Programme" launched in 1958 although Puri (1955) and Peters et al. (1955) were the first to report some species of *Anopheles*, and Peters and Dewar (1956) to record certain culicine species. Stone, Knight and Starcke in the "Catalogue of the Mosquitoes of the World, 1959" incorporated 17 anopheline and 19 non -anopheline mosquitoes from Nepal. In 1960 Pradhan and Brydon added one, *Anophelese filipinae* a native of Philippines, in the list from Kaski District. Brydon et al. (1961) reported 31 anopheline species as a vector of malaria from central Nepal. Joshi et al. (1964) added two more anopheline mosquito (*A. kochi*, *A. balabacensis*) of the *Leucosphyrus* group from Jhapa. Thereafter, in 1966 Shrestha made an extensive review of the mosquitoes of Nepal and reported a total of 97 species including 36 anophelines and 61 culicines. Before this Joshi, Pradhan and Darsie in 1965 listed 59 nonanopheline species (Culicine, Sabethine and Toxorhynchiline) from twenty-two districts of Nepal. It included three more genera - *Culiseta* Felt, *Malaya* Leicester and *Topomyia* Leicester, four sub - genera and 28 species mostly collected from the Terai, inner Terai and not above 1,300m. Then Jung (1973) reported *Culex fatigans* Wiedmann as predominant and vector of *Wuchereria bancrofti* (a filarial nematode) in various places from central-north Barabise (Kavrepalanchok District) to South Birjung (Parsa District), and during the epidemic of Japanese viral encephalitis in 1978 few specimens of culicines were collected from Jhapa, Morang, Sunsari, Parsa and Rupandehi

Districts and later confirmed by Joshi. This then added two more species, *Armigeres flavus* and *A. obturbans*.

Similar records of anopheline mosquitoes from Nepal were made by Ramachandran Rao (1981). No new additions were made until 1989 when Pradhan and Darsie published 13 new records for Nepal and in 1990 they reported 7 more species (*Aedes pulchriventer*, *A. subalbopictus*, *Anopheles fragilis*, *A. dravidicus*, *Armigeres durhami*, *Culex infula* and *C. pseudovishnui*). Darsie and Pradhan (1990) have prepared a list of 130 species and subspecies in 14 genera of mosquitoes distributed in different parts of Nepal.

In 1990, Darsie and Pradhan published an extensive account of the mosquitoes of Nepal, recording 130 species in 14 genera. A total of 24 species of *Aedes* mosquitoes were reported from Gandaki, Janakpur, Mahakali, Seti, Koshi, Bagmati, Narayani and Karnali Zones of Nepal. This genus has been reported from low to high altitudes of Jumla district. The recorded 45 species of *Anopheles* mosquitoes are distributed from the Terai and inner Terai areas to the hills and mountains of the country. Among 45 *Anopheles* species only seven have been reported as malaria vectors of primary importance. These include: *Anopheles minimus*, *Anopheles fluviatilis*, *Anopheles annularis*, *Anopheles maculatus*, *Anopheles dravidicus*, *Anopheles pseudowillmori*, and *Anopheles willmori* (7-8). Similarly, 9 species of *Armigeres* distributed in Bagmati, Lumbini, Narayani and Koshi zones of Nepal. After *Anopheles* another largest genus of mosquito distributed in Nepal is *Culex* with 29 recorded species. Like *Anopheles*, *Culex* are also distributed throughout the country except high mountains but has been reported in the high altitudes of Jumla from various habitats. Over the last decades, deforestation and effective malaria control program using Dichloro-Diphenyl Trichloroethane (DDT) eliminated *An. minimus* during 1960s [5]. *An. fluviatilis* is now the primary malaria vector in Nepal, *An. annularis* the secondary malaria vector and the *An. maculatus* complex members are seasonal malaria vectors in the mountain region of Nepal [6-9].

Malaria has remained as a major public health problem impacting on the health and lives of a large proportion of people particularly in remote areas with low economies and concentrated along international borders [10-11]. Entomological surveillance refers to the regular, systematic collection, analysis and interpretation of entomological data for risk assessment, planning, implementation, monitoring and evaluation of vector control interventions. All surveillance activities must be clearly linked to programme decisions to

ensure optimal vector control [11]. Effective vector control is reliant on knowledge of local vector species and their susceptibility to insecticides, as well as on vector and human behaviours that may allow mosquitoes to avoid contact with interventions and thereby maintain residual transmission [12]. Periodic collection of such data is essential to inform vector control strategies and track their impact on malaria transmission. A number of methods are available for detecting or monitoring immature and adult *Anopheles* population. Selection of appropriate sampling methods depends on surveillance objectives, levels of infestation, available funding and skills of personnels [13]. Nepal's National Malaria Strategic Plan (2014-2025) has since endorsed surveillance as a core intervention to reduce and ultimately interrupt malaria transmission.

In Kailali, malaria displays geographical heterogeneity and is exemplified by the so-called border malaria type, with most of the malaria cases concentrated along the border of Nepal-India. Malaria transmission along the Nepal- India border area is high because of extensive population movement across the border, especially mobile and forest workers, who make a substantial contribution to the regional malaria burden. The vectorial capacity and relative importance of these vector species in malaria transmission are, however, poorly understood, hence representing a threat to the success of malaria control and elimination in this region. The survey is aimed to investigate the species composition of *Anopheles* species in Bardagoriya-4, Kailali district.

1.2. Objectives of the study

The major objectives of this study were:

- To find out species composition and abundance of *Anopheles* species.
- To study the bionomics of proven malaria vector *Anopheles* in the study area.

1.3. Rationale of the study

Malaria is one of the major public health problem in Sudurpaschim Province since long time. The open borderline connection with India, increasing trend of human population, destruction of forests for urbanization, negative impact of climate changes, lack of prompt health facilities and social awareness and so on have led to higher risk of vector borne diseases transmission such as malaria. Early diagnosis of malaria and proper treatment along with systemic malaria vector control strategies are the foremost necessities to achieve the target of zero indigenous malaria cases by 2022 and malaria eliminated Nepal by 2025. Bardagoriya Rural Municipality, ward 4 of Kailali district was selected for integrated vector surveillance due to incidence of indigenous malaria cases in recent years. Sudurpaschim Health Directorate has recorded 37 (Shrawan 2075- Ashar 2076), 5 cases (Shrawan 2076- Ashar 2077) and 2 indigenous malaria cases (Fiscal year 2077/2078) in Kailali District. This study provides the baseline information about the malaria vector *Anopheles* in Kailali. This study also helps to strengthen the malaria vector control programmes, operational activities and further entomological surveillance researches.

2. MATERIALS AND METHODS

2.1. Study Area

Kailali district of Nepal is located in far-western Province-7 having the spatial extent between latitude $28^{\circ} 23' 36.34125''$ N to $29^{\circ} 4' 8.37569''$ N and longitude from $80^{\circ} 28' 2.64791''$ E to $81^{\circ} 17' 20.71635''$ E covering an area of about 3,235 sq. km as shown in Figure 1. The elevation of the district varies from 109 meters in the Terai belt to 1950 meters in the mountainous areas. Nearly 60% of the study area have elevation below 300m, making it lower tropical climate; around 26% area falls under upper tropical climate having the elevation range between 300-1000 m; and the mountainous areas (1000 -2000 m) falling under sub-tropical climate covers nearly 14% of the study area [24]. Usually, the district has a mild and warm temperature that varies from a maximum of 43° C in summer and a minimum of 5° C in winter season with an average annual temperature of 31° C. Around 65% of the district is covered by vegetation/ forest and 28% is covered by cultivated land [24]. The district has a total population of about 775,709 in 2011 with 2.29% population growth rate [23]. The region has complex socio-economic structures. The people with lower income have small houses made up of muds and bricks, mixed type of dwelling habits with cattle sheds facing the problem of moderate to high risk of vector borne diseases transmission such as malaria. Agriculture is one of the main occupation for the source of their income and some of them have also migrated to India in the quest of employment.

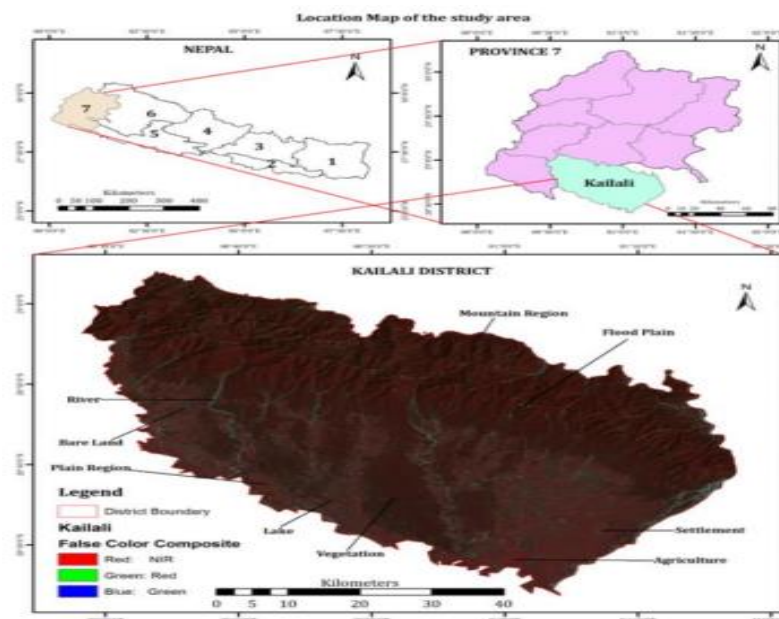


Figure 1: Study area [Sources: KC et al. 2020]

2.2. Materials

1. Global Positioning System (GPS) device
2. Sucking Tube/ aspirators
3. Test Tubes
4. Vials and droppers
5. Forceps
6. Petri dish
7. Cotton, Mask, Gloves
8. Silica gel, Chloroform.
9. Eppendorf tubes
10. Hand Torch
11. Stereoscope

2.3. Study Design, Populations, and Sampling Methods

Entomological surveillance was conducted from 2078/01/24 to 2078/01/30 in Bardagoriya-4, Kailali. In the study area, a committee composed of public health officers, vector inspectors, village leaders, village malaria workers, and volunteers was formed to assist and to organize the surveys and in engaging and mobilizing the community. Informed consent was obtained directly from participants. Adult female *Anopheles* mosquitoes were collected by using aspirators inside and outside of animal sheds as well as human residence during day and night. Morning indoor collection was performed between 5.30am to 7.30am. Likewise, animal night biting (intra and extra), animal resting as well human biting collection were carried out from 6 pm to 6 am. At the time of collection, female *Anopheles* as well as other vectors such as *Culex* spp. and *Phlebotomus* spp. were captured by aspirators. The collected samples were taxonomically identified using valid identification keys and preserved in Eppendorf tubes with silica gel for further study.

2.4. Data Analysis

All the collected data of this entomological survey was arranged in Ms. Excel and further analysed in order to find out *Anopheles* abundance, species composition and their bionomics.

3. RESULT

3.1. Species composition of *Anopheles* in the study area

Four species of *Anopheles* were recorded out of total 118 female *Anopheles* specimens during morning hand collection. The most abundant species was *An. annularis* (42.37%) followed by *An. culicifacies* (39.83%), *An. subpictus* (16.95%) and *An. splendidus* (0.85%) from Bardagoriya-4 during this study.

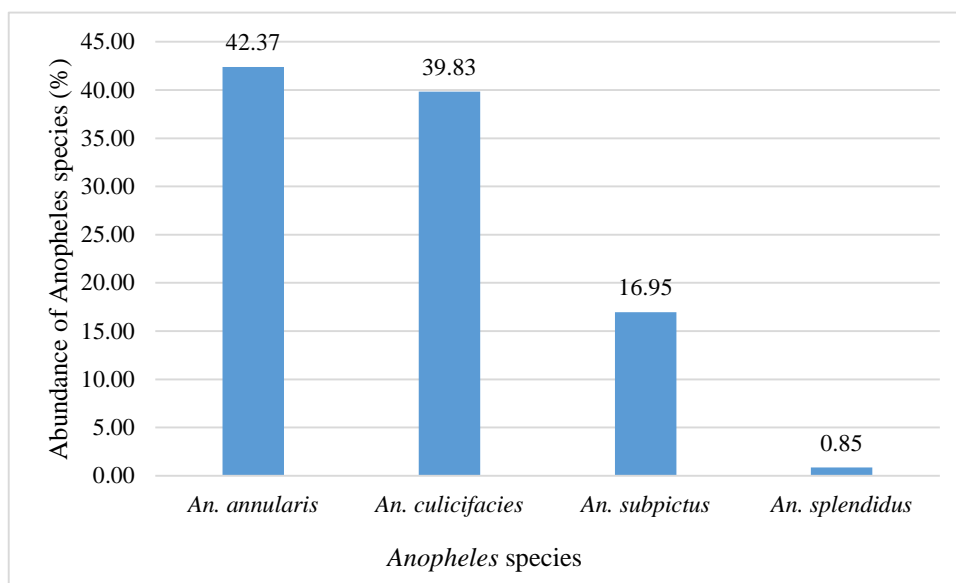


Figure 2: *Anopheles* abundance during morning hand collection in Bardagoriya-4, Kailali

Similarly, while observing the abdominal condition of 118 collected female *Anopheles*, high proportion of specimens were freshly fed (43.22%) followed by half gravid (37.29%) and gravid (19.49%) during the morning indoor hand collection.

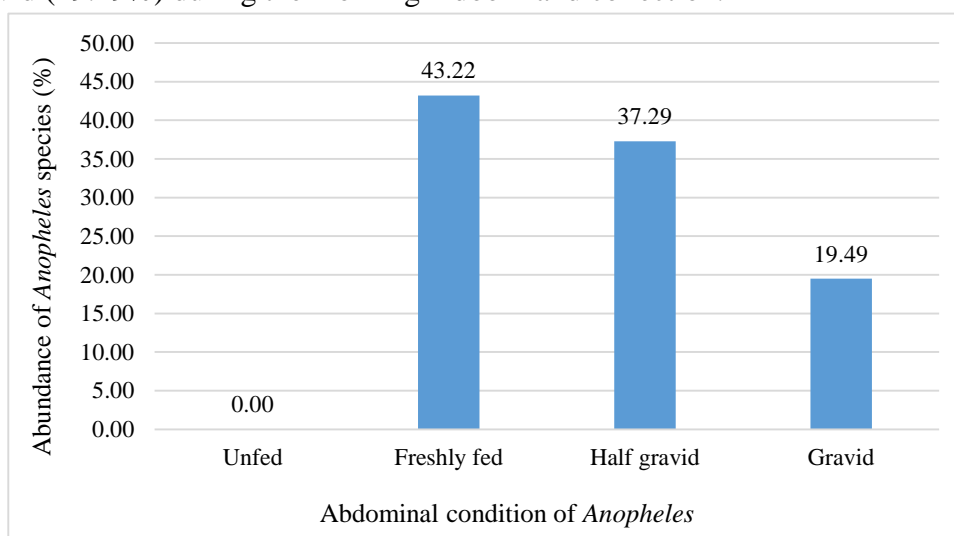


Figure 3: Abdominal conditions of *Anopheles* species during morning indoor hand collection, Bardagoriya-4, Kailali

3.2. Species composition of *Anopheles* species during night collection

Out of total 86 collected *Anopheles* specimens, four species were identified representing *An. annularis* (36.67%), *An. subpictus* (30%), *An. culicifacies* (28.89%) and *An. splendidus* (4.44%) during night collection in Bardagoriya-4.

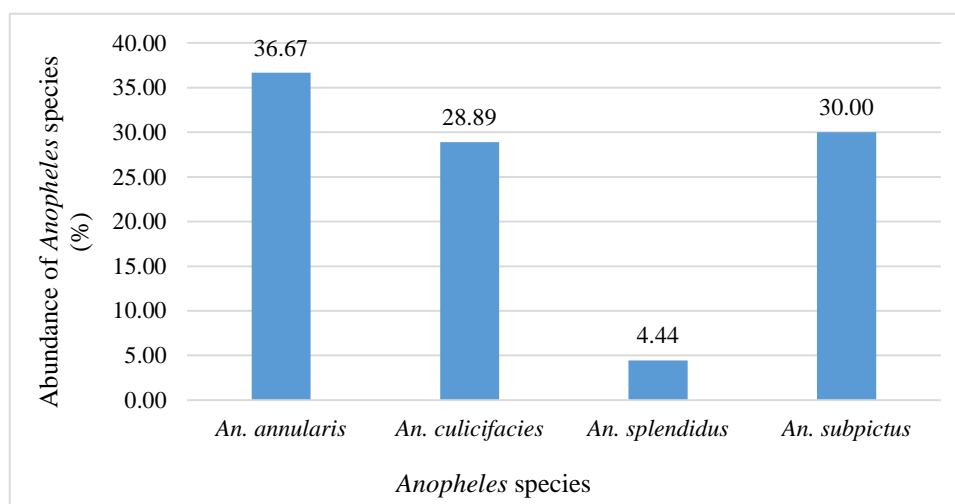


Figure 4: Abundance of *Anopheles* during night collection in Bardagoriya-4, Kailali

During this study, the highest number of *Anopheles* was captured by Animal resting Indoor method (n=39) followed by Outdoor Animal Biting method (n=27), Indoor Animal biting method (n=20) and only 4 *Anopheles* individuals were captured by Outdoor Human biting methods. No *Anopheles* were collected by Indoor human biting collection method and Natural outdoor collection method. Besides the *Anopheles*, other vectors such as *Culex* (n=126) individuals were also captured during the night collection in th study area.

Table 1: The density of *Anopheles* species during night collection in the study area

District: Kailali Location: Bardagoriya Rural Municipality, Ward 4								
		Types of collection methods during night sampling						
S.N.	Collected <i>Anopheles</i> species	Animal biting(indoor)	Animal biting (outdoor)	Animal resting (indoor)	Human Biting (indoor)	Human Biting (outdoor)	Natural outdoor	Other vector
1	<i>An. annularis</i>	7	10	14	0	2	0	Culex= 126
2	<i>An. culicifacies</i>	6	7	12	0	1	0	
3	<i>An. splendidus</i>	2	1	1	0	0	0	
4	<i>An. subpictus</i>	5	9	12	0	1	0	
	Total	20	27	39	0	4	0	

3.3. Bionomics of identified vector species in the study area

The study has shown that *An. annularis*, *An. culicifacies*, *An. subpictus* and *An. splendidus* were collected from mixed dwellings mostly in fully fed or gravid representing both zoophilic as well as anthropophilic behavior in Bardagoriya Rural Municipality-4. *Anopheles* specimens were seldom captured in outdoor shelters and human bait in the sampling site. Apart from the collection of *Anopheles* species, other vector species i.e. *Culex* spp. were captured using human as bait during night collection. It was found that higher density of *Anopheles* as well as other vector species were collected during early morning and early evening hour than during day or midnight during our study. However, there is variation in the feeding and resting behavior of *Anopheles* depending on their species. Thus, this study emphasizes in further exploring and understanding the bionomics of various *Anopheles* species in all of lower, moderate and highly risk malaria endemic areas in order to implement an effective malaria vector control programme.

Table 2: Vector density in the study area

District: Kailali									
Location: Bardagoriya Rural Municipality (Ward 4)									
Type of collection	Vector species	No of individuals			Abdominal conditions				Other vectors
		Male	Female	Total	Unfed	Full fed	Half gravid	Gravid	
Morning Indoor hand collection	<i>An. annularis</i>	0	50	50	0	31	12	7	
	<i>A. culicifacies</i>	2	47	49	0	16	23	8	
	<i>An. subpictus</i>	1	20	21	0	3	9	8	
	<i>An. splendidus</i>	0	1	1	0	1	0	0	
	Total	3	118	121	0	51	44	23	
Night collection	<i>An. annularis</i>	0	31						Culex= 126
	<i>An. culicifacies</i>	0	25						
	<i>An. splendidus</i>	0	4						
	<i>An. subpictus</i>	0	26						
	Total	0	86						

3.4. Taxonomic characteristics of identified *Anopheles* species (Darsie and Pradhan, 1990):

1. *An. annularis* Vander Wulp, 1884

- Wing with 4 or more dark spots on costa, involving costa and vein R, wing never all dark-scaled (subgenus *Cellia*)
- Femora and tibiae not speckle
- Some or all of hind tarsomeres 3-5 pale-scaled
- Hind tarsomeres 3-5 pale-scaled
- Wing vein Cu mainly dark-scaled, with dark spot at bifurcation of Cu, and Cu

2. *An. culicifacies* Giles, 1901

- Wing with 4 or more dark spots on costa, involving costa and vein R, wing never all dark-scaled (subgenus *Cellia*)
- Femora and tibiae not speckle
- Hind tarsomeres 3-5 not pale-scale
- Fore tarsomeres entirely dark-scaled, or with very narrow pale bands
- Palpomere 5 pale-scale
- Vein R with dark spot opposite humeral cross vein, wing vein R4+5 mainly dark scaled

3. *An. subpictus* Grassi, 1899

- Wing with 4 or more dark spots on costa, involving costa and vein R, wing never all dark-scaled (subgenus *Cellia*)
- Femora and tibiae not speckle
- Hind tarsomeres 3-5 not pale-scaled
- Fore tarsomeres with broad, pale-scaled band
- Subapical dark band on palpi usually more than 0.33 length of apical pale band; proboscis usually all dark-scaled
- Subapical pale band on palpi 0.33 or less length of subapical dark band; subapical dark band more than 0.5 length of apical pale band

4. *An. splendidus* Koidzumi, 1920

- Wing with 4 or more dark spots on costa, involving costa and vein R, wing never all dark-scaled (subgenus *Cellia*)
- Femora and tibiae speckled with pale and dark scale
- Some or all of hind tarsomeres 3-5 pale-scale
- Hind tarsomere 5 completely pale-scaled; abdominal sterna without scale tufts; palpi with 3 distinct pale bands, including apical band
- Hind tarsomere 5, all of 4 and at least part of 3 completely pale-scale
- Hind tarsomeres 3-5 completely pale-scale
- Palpi speckled, apical and subapical pale-scaled bands equal in length

4. DISCUSSION

Maharjan et al. (2014) has recorded 21 species of mosquitoes belonging to five different genera from Kathmandu, Rupandehi, Kapilbastu and Morang districts. Distribution of *Culex* mosquitoes was found in the entire district with highest density *Culex quinquefasciatus* in Kathmandu and Morang. Density of *Culex tritaeniorhynchus* was found high in Rupandehi district compared to other district. Both distribution and density of the *Culex* was higher in Rupandehi and Kapilbastu as compared to eastern region of Nepal in their study. They have reported eight different species of *Anopheles* i.e. *Anopheles vagus*, *An. sinensis*, *An. jamesii*, *An. annularis*, *An. maculatus*, *An. fluviatilis*, *An. culicifacies* and *An. nigerrimus* with a prevalence of 4.9%, 1.7%, 0.06%, 0.13%, 0.06%, 0.06%, 0.13% and 0.59% respectively. Three species of mosquito belonging to genus *Aedes* includes *Ae. vittatus*, *Ae. albopictus* and *Ae. aegypti* with prevalence rate 0.19%, 0.32% and 0.19% respectively has also been reported only from the Kapilbastu and Rupandehi districts. Two species of *Mansonia* mosquito were collected from Kapilbastu district with less density while only *M. uniformis* was reported from Morang district. Besides, Kapilbastu district, *Armigeres kuchingensis* has been reported from all three district of the country with overall prevalence (1.3%) [14].

The risk of mosquito-borne diseases has put world under threat for long years. Many dependent variables, particularly physical and biological parameters of the breeding site directly influence the species richness and abundance patterns of mosquito species. Hot and humid climate, long rainy season and abundant diverse mosquito breeding habitat are some of the important factors which influence the high prevalence of the vector borne diseases. [15]. The favorable mosquito breeding areas vary from genus to genus and species to species. The *Anopheles* mosquito generally prefers to breed in slow flowing streams and rivers [16,17]. The grasses that grow abundantly near the slow flowing streams offer favorable breeding grounds for colonization of *Anopheles* mosquito. Similarly, paddy fields are suitable sites for vector breeding during monsoon seasons [18]. During this study, higher abundance of *Anopheles* was observed from mixed dwellings with poor sanitation, houses having fish ponds, ditches and water resources suitable for mosquito breeding. The presence of *Anopheles*, *Culex* as well as sandflies has been one of the major threatening indicator of transmission of several vector borne diseases in those areas.

Malaria is also known as poor man disease and the poor new settlers' clears large part of reserved forest over a period of time for acquiring land for agricultural activities [19]. They are also actively associated with illegal forest wood smuggling, which encountered them with *Anopheles* mosquitoes while staying in the jungle. The severity of the disease and its association with deforestation is well established by Nath et al. (2012) [20]. As such the presence of malaria mosquito vectors and both symptomatic and asymptomatic people around such mosquito vectors in suitable environmental conditions increases the risk of disease in the community. However, a lack of breeding sites for the *Anopheles* mosquitoes can lead to less frequent disease transmission [21].

Dhimal et al. (2014) also documented the seasonal occurrence and altitudinal distribution of the vectors of other major VBDs in three districts (Morang, Dhankuta and Terhathum) of eastern Nepal. The altitudes of the study sites varied from 70 m to 2,500 m asl. A total of 2,538 adult mosquitoes belonging to the four genera *Aedes* (10%), *Anopheles* (55%), *Armigeres* (6%) and *Culex* (29%) were collected in both seasons. Among the *Aedes* specimens (n = 245), the known dengue virus vectors in Nepal, *Aedes aegypti* and *Aedes albopictus*, constituted 3% and 4%, respectively. The principal vector of lymphatic filariasis, *C. quinquefasciatus*, and the Japanese encephalitis virus vector *Culex tritaeniorhynchus* accounted for 12% and 31% of the total collected *Culex* mosquitoes (n = 742). Similarly, the known malaria vectors in Nepal, *An. annularis*, *An. fluviatilis* and *An. maculatus* complex members, represented 16%, 9% and 10% of the total collected *Anopheles* mosquitoes (n = 1396). Hence, about 35% of the total *Anopheles* mosquitoes belonged to species that are known malaria vectors. All three known malaria vector species were found throughout the studied altitude range up to 1,820 m asl. Similarly, other disease vectors *A. aegypti*, *A. albopictus*, *C. quinquefasciatus* and *C. tritaeniorhynchus* were also collected throughout the studied transect ranging from 70 to 2,000 m asl. They also collected *Anopheles*, *Culex* and *Aedes* larvae up to 2,310 m in their study area [22]. Similarly, the presence of higher abundance of *An. culicifacies* and *An. annularis*. *An. splendidus* and *An. subpictus* in our study has also supported the wide range of distribution of mosquito vector species in Kailali district of Nepal which probably lead to the spread of vector borne disease transmission in this area.

5. CONCLUSION

The presence of such high proportion of potential vectors of malaria *An. annularis*, *An. culicifacies*, *An. splendidus*, and *An. subpictus* as well as such as *Culex* spp. has indicated a threat of vector borne diseases such as malaria transmission in Kailali. Most of the *Anopheles* mosquitoes were collected from human dwellings attached with cattle sheds having poor sanitation in villages. The presence of *Anopheles* in both types of habitats i.e. human houses and cattle sheds indicated the both zoophilic and anthropophilic behavior of *Anopheles* in that study area, thus enhancing the rate of malaria transmission especially among people living under poverty. During this study, it was informed by the villagers that the households were unprotected by insecticide residual sprays (IRS) since long and they have been suffering from mosquito bites especially during monsoon seasons. Hence, human and vector contact should be minimized throughout the year together with long-lasting insecticide-treated nets and insecticide residual sprays to reduce the malaria transmission. Early malaria diagnosis and treatment along with appropriate vector control strategies are required to lower the incidence of indigenous malaria cases and to meet the target of Malaria Elimination Program by 2025.

6. RECOMMENDATION

1. More information on vector ecology, demographic characteristics, and socioeconomic structure in the study villages are needed to better understand the factors associated with human-vector contact and malaria transmission.
2. Timely identification of population subsets at high risk of exposure to malaria vectors could help national malaria control programs to eliminate potential transmission sources.
3. Proper co-ordination among all the health workers, researchers, program planners and stakeholders should be maintained among all central, provincial and local levels to achieve the target of Malaria Elimination Program by 2025.

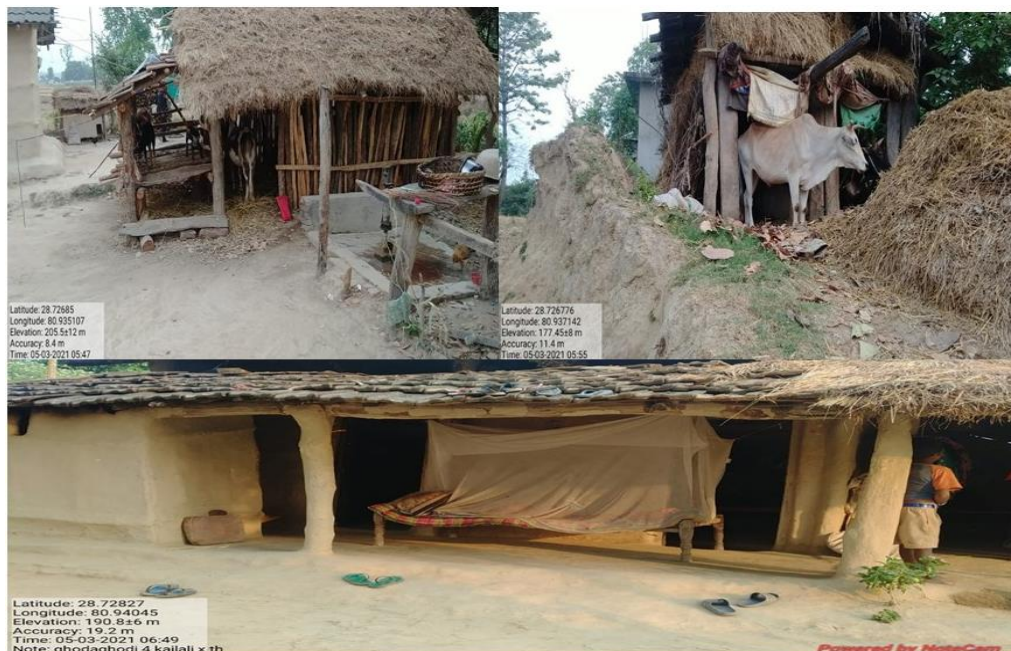
7. PHOTOGRAPHS OF THE ENTOMOLOGICAL SURVEY IN BARDAGORIYA RURAL MUNICIPALITY (WARD 4), KAILALI DISTRICT (2078/01/24-2078/01/30)



1. Outdoor Human Biting collection method



2. Light traps for night collection



3. Different habitats used for adult *Anopheles* collection



4. Different aquatic habitats used for larvae and pupae stages collection of *Anopheles* spp.



5. Collection of adult *Anopheles* spp. using aspirators



6. Identification of *Anopheles* spp.

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