Final Technical Report

for

Research Project

Entomological survey of major vector-borne diseases in geo-ecological and climatic variances in Province 1 of Nepal

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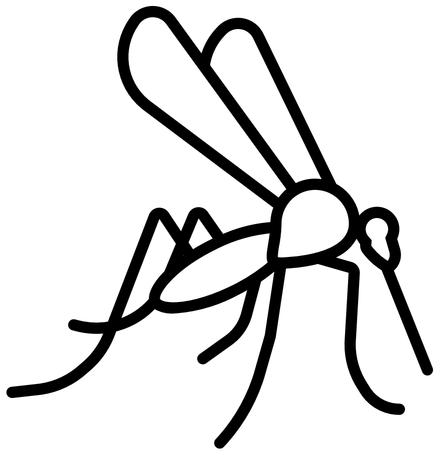
Entomological Survey of Major Vector-Borne Diseases in Geo-ecological and Climatic Variances in Koshi Province, Nepal



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Executive Summary

Nepal is broadly divided from north to south into three ecological zones: the high mountains, the hills and the lowland (also known as the 'Terai'). This topographical differentiation is well reflected in geography and sociocultural diversity. Despite its relatively small area, Nepal has very diverse climatic conditions, ranging from tropical in the south to alpine in the north. Nepal is home to an estimated 30 million people and more than 80% of the population lives in rural areas with a particular concentration in the lowlands.

Nepal is endemic to six vector-borne diseases viz. malaria, visceral leishmaniasis (VL), lymphatic filariasis (LF), Japanese encephalitis (JE), dengue, and scrub typhus. Pathogens of vector-borne diseases (VBD) are transmitted to humans through the bite of infected blood-feeding arthropods (vectors) like mosquitoes, sand flies or mites. Historically, the lowlands - which used to be covered by forest until the early 20th century - carried the major share of VBDs in Nepal. In recent years, VBDs have been spread to other previously non-endemic regions as well, including the hilly and mountainous regions. This geographical shift in the distribution of VBDs is attributed to several factors such as economic migration, changes in human behavior, and climate change. Besides, successive changes in temperature and precipitation are supportive of vector survival, transmission cycle, and, ultimately, wider geographical distribution. This shift of vector-borne diseases towards new areas may carry a risk for large-scale epidemics in relatively naïve susceptible human populations. Presence of major vectors like *Anopheles maculatus* complex and *An. annularis*, *Aedes aegypti*, *Ae. albopictus*, *Culex quinquefasciatus*, *Cu. tritaeniorhynchus* and *Phlebotomus argentipes* in this survey is evident to the threat of the local transmission of the VBDs in wide geo-ecological regions. The result is suggestive to the national VBDs control program for the systemic vigilance of the disease and vectors with suitable adaptation of disease control strategies including integrated vector management (IVM).

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Abbreviations and acronyms

BG-sentinel trap	Biogents-sentinel trap
BI	Breteau index
BPKIHS	B.P. Koirala Institute of Health Sciences
CBT	Cattle-Baited Trap
CDC light trap	Centre for Disease Control and Prevention light trap
CI	Confidence interval and Container index
CS	Cattle shed
HD	Human dwelling
HH	Household
HI	House index
IRS	Indoor Residual Spraying
IVM	Integrated Vector Management
JE	Japanese Encephalitis
LF	Lymphatic filariasis
MD	Mixed dwelling
MoHP	Ministry of health and population
NTDs	Neglected Tropical Diseases
PPP	Pupae per person
SOP	Standard operating procedure
VBDs	Vector-borne diseases
VBDRTC	Vector-borne disease research and training centre
VL	Visceral leishmaniasis
WN	Ward Number
WHO	World Health Organization

1. Background

Nepal is broadly divided from north to south into three ecological regions (Figure 1); the mountains, the hills and the lowland (also known as 'Terai'), and this topography is reflected by geographical and sociocultural diversity. The country is administratively divided into 7 provinces and 77 districts. Thought Nepal is well known for its mountains, more than 80% of the total population of 30 million lives in lowland and hosts most of the tropical and subtropical diseases.

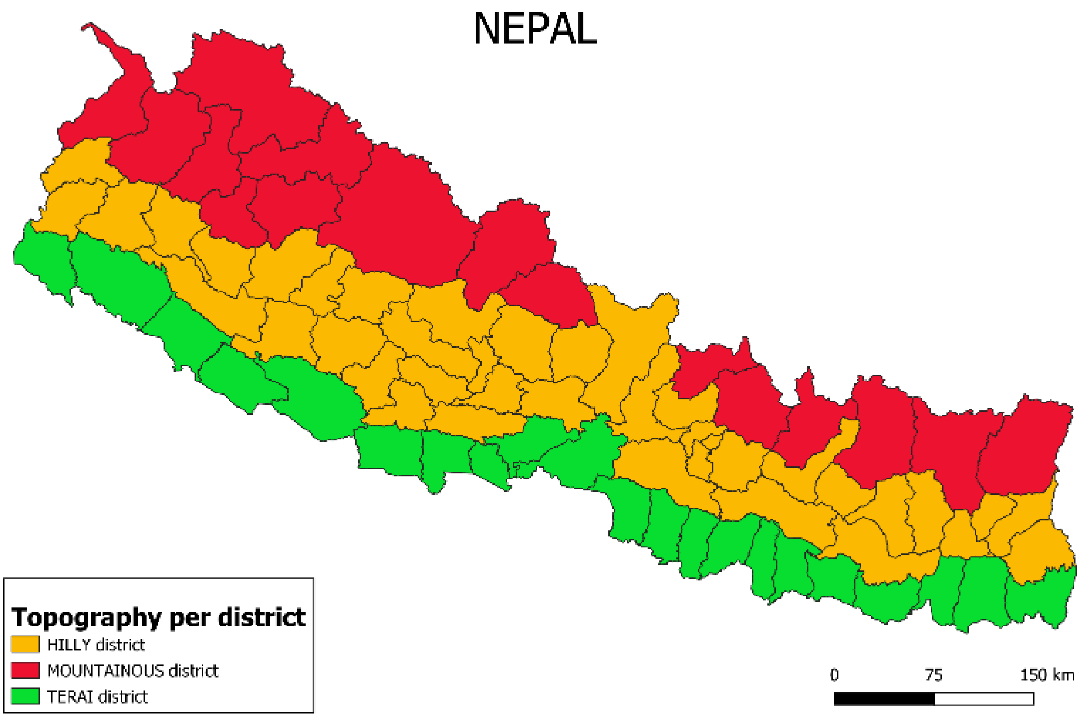

Figure 1.Topographical zones in Nepal.

Nepal is endemic to at least six vector-borne diseases; malaria, visceral leishmaniasis (VL), lymphatic filariasis (LF), Japanese encephalitis (JE), dengue and scrub typhus [1]. Vector-borne diseases (VBDs) are diseases caused by a pathogen which is transmitted to humans through the bite of infected blood-feeding arthropods (vectors), such as mosquitoes, sand flies or mites. Some of these VBDs such as VL, LF and dengue belong to the group of Neglected Tropical Diseases (NTDs), diseases which typically affect the poorest and most marginalized populations without receiving the adequate resources for good management. Historically, the lowland was mostly covered by forest until the early 20th century and many VBDs have been endemic to this region. However, in recent years, VBDs have spread to hills and mountains. These regions were once considered unsuitable to thrive the vector population and thus transmission of pathogen was not anticipated. This geographical shift in distribution of VBD is attributed to several factors, of which climate change is assumed to be the most important one. Changes in temperature and precipitation found to be supporting vector survival, maintaining the transmission cycle, and thus leading to a wider geographical distribution of the pathogens, vectors and diseases [2-5]. Spread of VBDs in new regions, ultimately imposes the threat of large-scale epidemics in relatively naïve susceptible populations, with overburdening of unprepared health systems and low-quality health care as a consequence [4, 6, 7].

Nepal has been experiencing noticeable changes in climate over the past decades especially in two crucial climatic variables: temperature and precipitation. Temperature increased with 1.5°C over the last two and a half decades, compared to 0.6°C at the global level [8]. Similarly, precipitation increased significantly with 5.3% per decade over the last six decades, with a more rapid increase since the mid-1980s [9]. Recently, changes in rainfall have been observed in Nepal. Rainfall increases with altitude on the windward side and sharply decreases on the downwind side in the middle Mountains. Seasonally, increasing trend of rainfall is seen in pre monsoon while decreasing trend is seen in monsoon, post monsoon and winter. Analysis of rainfall shows average annual rainfall is 1883.8 mm in lowlands (below 1000m elevation) and 1959.6 mm in highland (above 1000m elevation). July is the highest rain month and November is the lowest [10].

Several studies have shown a trend of increasing potential climatic indicators such as temperatures, rainfall and humidity in recent decades, with increasing warming trends in the hill and mountain regions. Due to the adaptation of climate sensitive vectors, the geographic expansion, outbreaks, and emergence of VBDs have been observed in high hills and mountainous as well [3].

2. Rationale of the survey

The major VBDs are climatic sensitive and climate change has been implicated as one of the potential factors for the expanding these diseases in previously not reported areas in recent time. Nepal is highly vulnerable to global climate change, urbanization, and its influence on health is inevitable. Malaria, VL, LF, JE, dengue and scrub typhus are the major important VBDs in Nepal. One of the current challenges in the control of these vector-borne diseases is to halt their emergence or expansion in new areas and at a higher altitude. Diseases like malaria, VL and LF are targeted for elimination by 2026 while JE, dengue and scrub typhus are projected for control in Nepal. In this context, surveillance of these diseases and their vectors is a key to contributing control of VBDs in Nepal and elsewhere.

There is sparse information available on the prevalence of vectors of the major VBDs at different climatic and ecological regions of Nepal. Lack of adequate vector surveillance data is considered as a major limitation in the proper planning and implementation of the control programme in Nepal. The needs for a comprehensive approach for vector control to counter the impact of VBDs have become more urgent and thus require information on the existing vectors for the implementation of integrated vector management (IVM). Integrated vector monitoring or surveillance is one step closer to the planning of IVM which has the economic benefit of vector control of major VBDs [11].

In this survey, we collected the baseline information on vectors of major VBDs in different geo-ecological and climatic variances in selected districts of Koshi Province in Nepal. The field work was anticipated to be laborious and time consuming as different approaches were required to collect various species of vectors. Hence, we selected only one province for piloting to check feasibility in terms of time, human resources and implementation of the integrated survey.

3. Objective of the survey

The main objective of the survey was to explore the vectors of major vector-borne diseases (malaria, VL, LF, JE, dengue, etc.) in different geo-ecological and climatic variances in selected districts of Koshi Province, Nepal.

The specific objectives were

- To document the vector abundances of major VBDs (and their distribution in different geo-ecological and climatic variances).
- To assess the relationship between the vector abundances and geo-ecological (mountain, hills, lowland, housing conditions,) and climatic factors (temperature, rainfall and relative humidity)
- To assess the spatial relationship between vector abundances and VBDs in the identified areas/districts.

4. Materials and methods

4.1 Study design

A descriptive cross-sectional survey in different a geo-ecological region was designed to explore the vector abundances and their distribution. The vector survey was conducted to assess the presence/absence of target vector species in selected clusters in three distinct ecological regions: mountain, hills and lowland.

4.2 Selection of survey districts

Epidemiological surveillance data of National VBDs of the Epidemiology & Disease Control Division, Department of Health Services, Ministry of Health and Population (MoHP), Government of Nepal was reviewed for the period of 2020 - 2022. Five districts from Koshi province were chosen based on recommendations of the provincial health officials to reflect differing climatic conditions and the following selection criteria.

- Districts should represent all three ecological regions: mountain, hills and lowland
- Reported of at least one of the any VBDs from the districts in the recent years (2020-2022).
- No baseline information available or poor knowledge of the vector abundances from the districts

Based on the above selection criteria, five districts namely Morang, Sunsari, Udayapur, Okhaldhunga and Sankhuwasabha were selected for the integrated vector survey. These selected five districts are representative of the three ecological regions: Morang and Sunsari districts in the lowland, Udayapur district in the low-mid hill, Okhaldhunga district in the high hill and Sakhunwasabha district in the mountainous region.

4.3 Selection of Survey clusters

In each district, two clusters (small hamlet or village) were selected primarily based on the reported cases of any of the one VBDs in recent years (since 2020-22) and have good access to transportation. The details of the survey clusters are given in Table 1 and their location in Figure 2.

Table 1. Details of the survey districts/clusters, Koshi Province, Nepal.

SN	Districts	Ecological region	Survey clusters	VBDs
1	Morang	Lowland	Rangeli WN-6 (Godhi tole)	VL
			Pathari Sanischare WN-7 (Mayalu chowk)	Malaria
2	Sunsari	Lowland	Dharan WN-8	Dengue

			Dharan WN-15	Dengue
3	Udayapur	Hills	Triyuga WN-6 (Deudi Purano tole)	VL
			Chaudandigadhi WN-10 (Devdhar)	VL
4	Okhaldhunga	Hills	Manebhanjyang WN-5 (Fedigaun)	VL
			Manebhanjyang WN-6 (Sokmatar)	VL
5	Sankhuwasabha	Mountain	Khandbari WN-9 (Sanguritole)	VL
			Chainpur WN-10 (Makpa)	VL

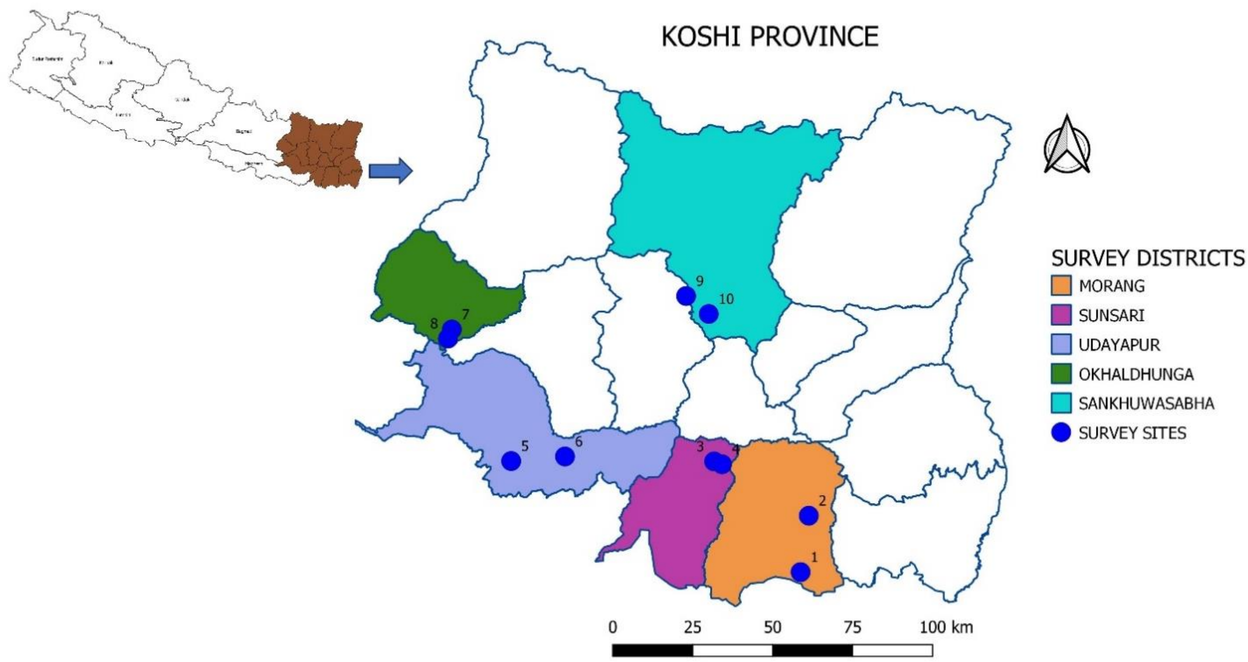

Figure 2. Location of vector survey districts and clusters in Koshi Province, Nepal, 2023

4.4 Fieldwork and vector collection

The integrated vector survey took place in Morang, Udayapur and Okhaldhunga districts in May and in Sankhuwasabha and Sunsari districts in June 2023. Timing of the insect collections (May-June) purposively coincided with the first annual peak of vector density in the lowland.

In each cluster, 10 households' structures (human dwelling, mixed dwelling, and cattle sheds) and nearby areas were explored for vector collection. In each selected household structure, mosquitoes and sand flies were captured for two consecutive nights by trained insect collectors under direct supervision of a medical entomologist. Adult and immature stages of vector and other non-vector species were also collected through various methods described below.

A. Adult vector survey

i. Adult *Anopheles* and *Culex* mosquitoes (vectors of the malarial parasite, LF, and JE) were collected both from indoor and outdoor human dwellings, cattle sheds, and mixed dwellings (where human beings and animals share the same roof in a structure) by using mouth aspirators. A cattle-baited trap (CBT) was used for outdoor mosquito collection at outside the household and/or nearby vegetation areas. Centre for Disease Control and Prevention (CDC) light traps were also used for indoor mosquito collection.

ii. *Aedes* mosquitoes (vectors of dengue, chikungunya and zika virus): Five Biogents (BG) sentinel traps were placed outdoors near the households and the vegetation for the collection of *Aedes* mosquitoes.

iii. Sand flies (vectors of kala-azar): CDC light traps were placed in indoors in human dwellings and mixed dwellings for sand fly collection. Resting sand flies were also collected by mouth aspirators in the early morning during the survey. In each selected cluster, 10 CDC light traps were fixed in 10 selected households, one in each of the houses or cattle sheds, for indoor mosquito and sand fly collections. Light traps were installed and operated from 18h the evening to 6h the next morning. The same process was repeated for the next day as well to have two consecutive nights of collection. Similarly, the resting sand flies were collected for two consecutive mornings by mouth aspiration for 15 minutes in the same households and the cattle sheds where LTs were installed. Mosquitoes and sand flies were collected and dry preserved in tubes with silica gel labeled with information on cluster, site and method of collection and transported to the entomology laboratory at BP Koirala Institute of Health Sciences, Dharan. Species-level identification of mosquitoes and sand flies were done using regional keys [12-20], stereoscope and light microscopes. After identification, mosquitoes were dry preserved in tubes with silica gel while sand flies were preserved in tubes with 80% ethanol at species and sex levels for each cluster.

B. Immature stage survey

Immature stages of mosquitoes (larvae and pupae) were collected from the possible breeding sites near the households in the selected clusters using larval sampling techniques such as dipping, netting and pipetting. A detailed survey targeting the immature stages of *Aedes* mosquitoes were conducted in two wards of Dharan sub-metropolitan city with rising cases of dengue fever in recent months during the survey. Larval and pupal sampling methods were adapted from SOP and guidelines developed by WHO [21, 22]. Random houses in the wards and public places were searched indoors and outdoors for the water holding containers and the presence of mosquito's immature stages in them. Overhead tanks were not searched due to inconvenience and safety reasons. Besides the water-holding containers for household purpose, the discarded plastic and metal containers, tyres, flower vases, plates kept under flower pots, kitchen gardens, mud pots, gallons, tree holes wherever possible, and any form of utensils that can hold water were searched for presence of immature stages. Positive containers were sampled and immature stages (larvae and pupae) were collected and transported to the entomology lab for further rearing to adult stage and then identified to species level.

C. Housing characteristics and geo-ecological information

GPS location of all surveyed households with latitude, altitude and elevation was recorded during the survey. Characteristics and feeding and resting sites of mosquitoes and sand flies were collected using a semi-structured questionnaire (Annex – 1). Information on details of housing structures, presence of cattle and other domestic animals, surrounding vegetation, water bodies, etc. were collected (Annex – 2) for the household where CDC LTs were kept. Characteristics of mosquito breeding sites including the description of water conditions (temporary/permanent, clear/turbid, stagnant/running, and sunny/shaded, presence/absence of vegetation) were also collected (Annex – 3 and Annex – 4).

D. Weather information

Potential important climatic variables such as daily records of rainfall, relative humidity (%), maximum temperature (°C), and minimum temperature (°C) were collected from the meteorological stations located <10 kilometers from the survey villages/clustered in the lowland and <5 kilometers from the survey clusters visited in upland (hills) for the period of one year (July 2022 – June 2023). These weather data were collected from the nearest meteorological stations of the Department of Hydrology and Meteorology, Government of Nepal.

The research field team was closely supervised by an epidemiologist and entomologist. The overall supervision of the activities has been made by the expert team from Vector Borne Disease Research and Training Centre (VBDRTC), Hetuada, Nepal.

5. Data management and analysis

The collected questionnaire /data were double-checked and verified for quality, completeness and accuracy by the entomologist and epidemiologist. The data was then entered into databases made in Epi Info version 3.5.1. All data files were checked and cleaned by the epidemiologist and entomologist before analysis. Objective-wise data analysis is described below.

Analysis for objective 1: To document the vector abundances of major VBDs (and their distribution in different geo- ecological and climatic variances).

Descriptive analysis of the climatic data (temperature, rainfall and relative humidity) has been presented in mean and monthly trend line graph was plotted according to the survey districts. Descriptive analysis of vector and non-vector species are performed and represented as described below. Abundances and species richness (S) of vector and non-vector species were represented by absolute numbers. Species diversity and dominance or uniformity in distribution of the species at district level were represented in Shannon-Wiener diversity index (H') and Pielou's evenness index (J) [23, 24]. H' and J are calculated using function 'diversity' from a R package "vegan" in R [25]. Mathematical calculation of these indices are:

(a) Shannon-Wiener diversity index $(H') = -\Sigma pi * \ln(p_i)$

Where, Σ: Sum, ln: Natural log and $pi = n_i/N$ (n_i = the number of individuals of a species and N = Total number of individuals)

(b) Pielou's evenness index $(J) = H'/\ln(S)$

Where, H' = Shannon-Wiener diversity index and S is the total number of species in a sample

Interpretation of Shannon-Wiener diversity index (H') was done as the higher the value of H', the higher the diversity of species and lower the value, the lower the diversity and if value of H' is 0 then only one species is present in that community. Pielou's evenness index (J) ranges from 0 to 1. Higher the value of J, higher the level of evenness in the abundance of different species present in a particular community while lower value represents either one or only few species are present in abundance. A landscape map was prepared to illustrate the relative abundances (proportion) of the vector species according to the survey districts and elevation.

A descriptive analysis of immature stages of mosquitoes, the habitat availability and their habitat occupancy were performed at district level. Similarly, *Stegomyia* indices (HH index, container index, Breteau index and pupae per person) were calculated for the immature stages of *Aedes* mosquitos collected from a detail investigation conducted in urban area of Dharan sub-metropolitan in Sunsari district.

Analysis for objective 2: To assess the relationship between the vector abundances and geo-ecological (mountain, hills, lowland, housing structure and surrounding conditions) and climatic factors (temperature, rainfall and relative humidity)

The vector abundance data was over dispersed and have shown non-normal distribution, i.e. variance was larger than mean value. Hence, we fitted generalized linear models (GLM) with a negative binomial distribution to assess the association of the vector abundance in function of the explanatory variables like ecological regions, method of collection, collection sites, climatic variables, household structures, and surrounding ecological features. Spearman's correlation was assessed between the each vector species with the mean temperature (°C), mean relative humidity (%) and cumulative rainfall (mm) of the preceding one month and the survey month, i.e. April and May 2023 before incorporating them in the model. Final model was fitted separately for each vector species. The vector species, *An. annularis, An. pseudowillmori, An. willmori, Cu. Tritaeniorhynchus, Ae. aegypti* and *Ae. albopictus* were not included in the final model due to their low number of collection. Hence, the model was fitted with two vector species with plausible collection; *Cu. quinquefasciatus* and *P. argentipes*. The calculation was done using the function 'glm.nb' from a R package "MASS" [26]. Results of the analysis are interpreted as an incidence rate ratio (IRR) and confidence interval (CI) at 95%.

Analysis for objective 3: To assess the spatial relationship between vector abundances and incidence of VBDs in the identified areas/districts.

Disease incidence rates for lymphatic filariasis and visceral leishmaniasis were calculated per 10,000 population at the district level from the available data and the national line list collected in 2022 and 2023. The incidence rate and vector abundance gradient map were constructed in QGIS ver 3.36. The association of the vector abundance and the presence of VBDs at the district level was analyzed with the method explained in objective 2. The outcome is explained in terms of IRR and confidence interval at 95%.

6. Ethical consideration

Ethical approval for the study was obtained from the Ethical Review Board of the Nepal Health Research Council (NHRC), Kathmandu, Nepal (268/2022P) and ethical review committee of the WHO South East Asia Regional Office, New Delhi, India.

7. Results

7.1 Characteristics of the survey districts, households in clusters and surroundings

The vector survey was conducted in 10 clusters from five districts namely Morang, Sunsari, Udayapur, Okhaldhunga and Sankhuwasabha. All districts are endemic for malaria, VL, LF. However, dengue is quite an emerging threat in all these districts. Altogether 100 households/household structures with human, cattle sheds and mixed dwellings were approached for vector survey.

The elevation of survey clusters ranged from 98m asl in one of the cluster in lowland district (Morang) to 1274 m asl in a cluster in high hill district (Okhaldhunga). The key characteristics of study districts, the selected households where light traps were kept and their surrounding areas are shown in Table 2.

Table 2: Key characteristics of the survey districts and households in Koshi Province, Nepal

General characteristics	**Morang**	**Sunsari**	**Udayapur**	**Okhaldhunga**	**Sankhuwasabha**
Ecological region	Lowland	Lowland	Low-mid hills	High hills	Mountain
Urbanization of survey clusters	Rural	Urban	Rural	Rural	Rural
Mean altitude of survey clusters (m asl)	98-139	309-323	437-623	1155-1274	832-1011
Endemic for VBDs	Yes	Yes	Yes	Yes	Yes
Household characteristics	**N = 20 (%)**	**N = 20 (%)**	**N = 20 (%)**	**N = 20 (%)**	**N = 20 (%)**
Type of Roof					
Cement	3 (15%)	0	0	0	0
Thatch (Straw and bamboo)	0	2 (10%)	1 (5%)	6 (30%)	9 (45%)
Tiles	0	0	1 (5%)	0	0
Tin	17 (85%)	18 (90%)	18 (90%)	14 (70%)	11 (55%)
Type of wall					
Cemented	8 (40%)	6 (30%)	4 (20%)	5 (25%)	1 (5%)
Unplastered brick	1 (5%)	2 (10%)	1 (5%)	2 (10%)	0
Mud	11 (55%)	10 (50%)	15 (75%)	13 (65%)	19 (95%)

Tin	0	2 (10%)	0	0	0
Type of Floor					
Cement	9 (45%)	10 (50%)	6 (30%)	5 (25%)	3 (15%)
Earthen	11 (55%)	10 (50%)	14 (70%)	15 (75%)	17 (85%)
Presence of ventilation	3 (15%)	7 (35%)	2 (10%)	4 (20%)	1 (5%)
Presence of cattle	7 (35%)	0	10 (50%)	12 (60%)	11 (55%)
Presence of cow dung	7 (35%)	3 (15%)	12 (60%)	15 (75%)	14 (70%)
Presence of goats	9 ((45%)	5 (25%)	9 (45%)	18 (90%)	14 (70%)
Presence of pigs	7 (35%)	2 (10%)	5 (25%)	4 (20%)	12 (60%)
Presence of agricultural field	18 (90%)	2 (10%)	18 (90%)	10 (50%)	18 (90%)
Presence of vegetable field	14 (70%)	7 (35%)	15 (75%)	14 (70%)	14 (70%)
Presence of mixed orchard	20 (100%)	13 (65%)	16 (80%)	18 (90%)	19 (95%)
Presence of River	10 (50%)	14 (70%)	18 (90%)	14 (70%)	6 (30%)
Presence of Pond	10 (50%)	1 (5%)	9 (45%)	6 (30%)	0
Mean number of domestic animals (± sd) per household					
Cattle	1.25 (±1.97)	0.05 (±0.22)	2.3 (±3.13)	2.5 (±2.40)	2.35 (±2.41)
Goats	2.05 (±3.3)	1.85 (±4)	1.95 (±3.89)	13.5 (±12.9)	4.2 (±5.32)
Pigs	1.15 (±2.23)	0.15 (±0.49)	0.5 (±1.1)	0.25 (± 0.55)	1.25 (±1.21)

7.2 Climatic variables

Temperature

The average daily maximum temperature of the surveyed clusters in three different ecological regions varied from 30.64°C in lowland (Sunsari district) to 23.02°C in high hills (Okhaldhunga district). The average daily maximum temperature was nearly 35°C in the month of June in lowland (Morang district) compared to 28°C in high hills (Okhaldhunga district) in the same month. So, there is already 7°C temperature variation in average daily temperature as well as average daily maximum temperature between lowland and highlands or hills. The average daily minimum temperature experienced in lowland (Rangeli, one of the cluster in Morang district) was about 8.84°C in January compared to 6.69°C in high hills (Okhaldhunga district) in the same month (Figure 3, 4, 5).

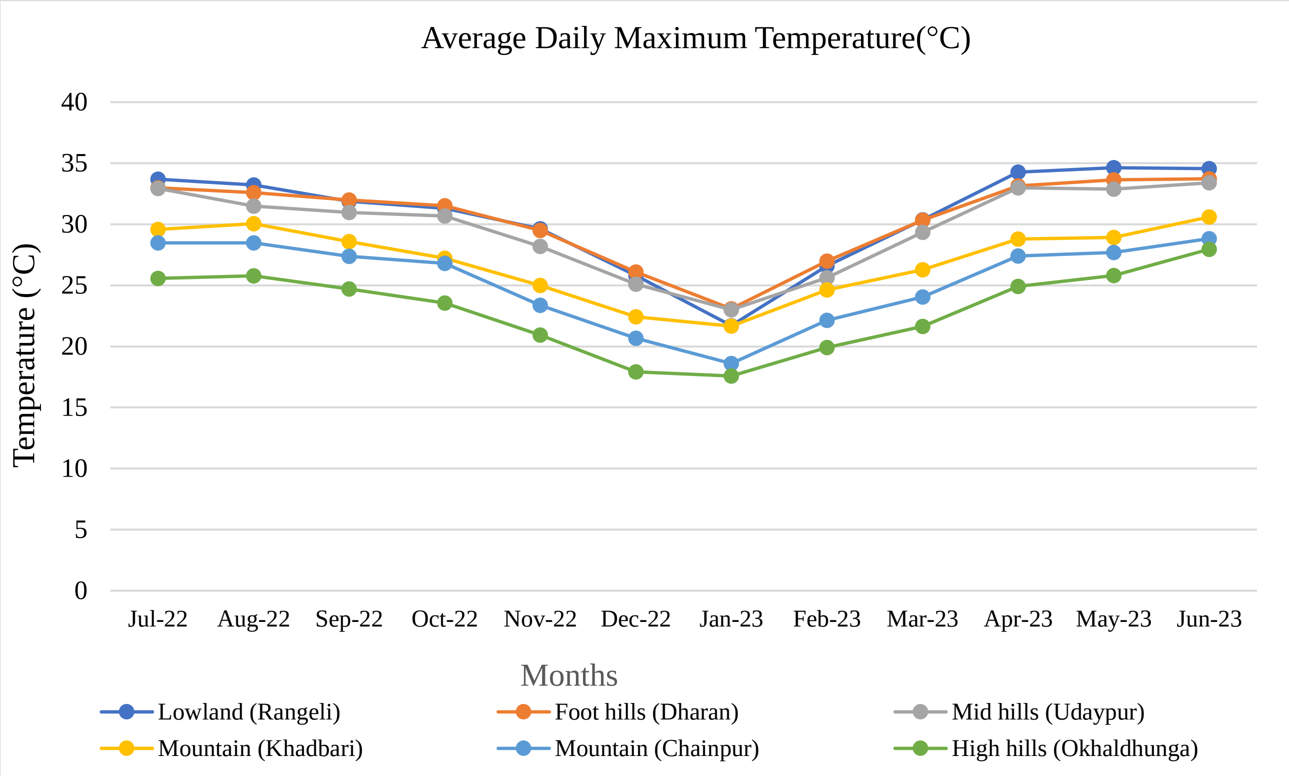

Figure 3. Average daily maximum temperature in survey districts (July 2022 – June 2023)

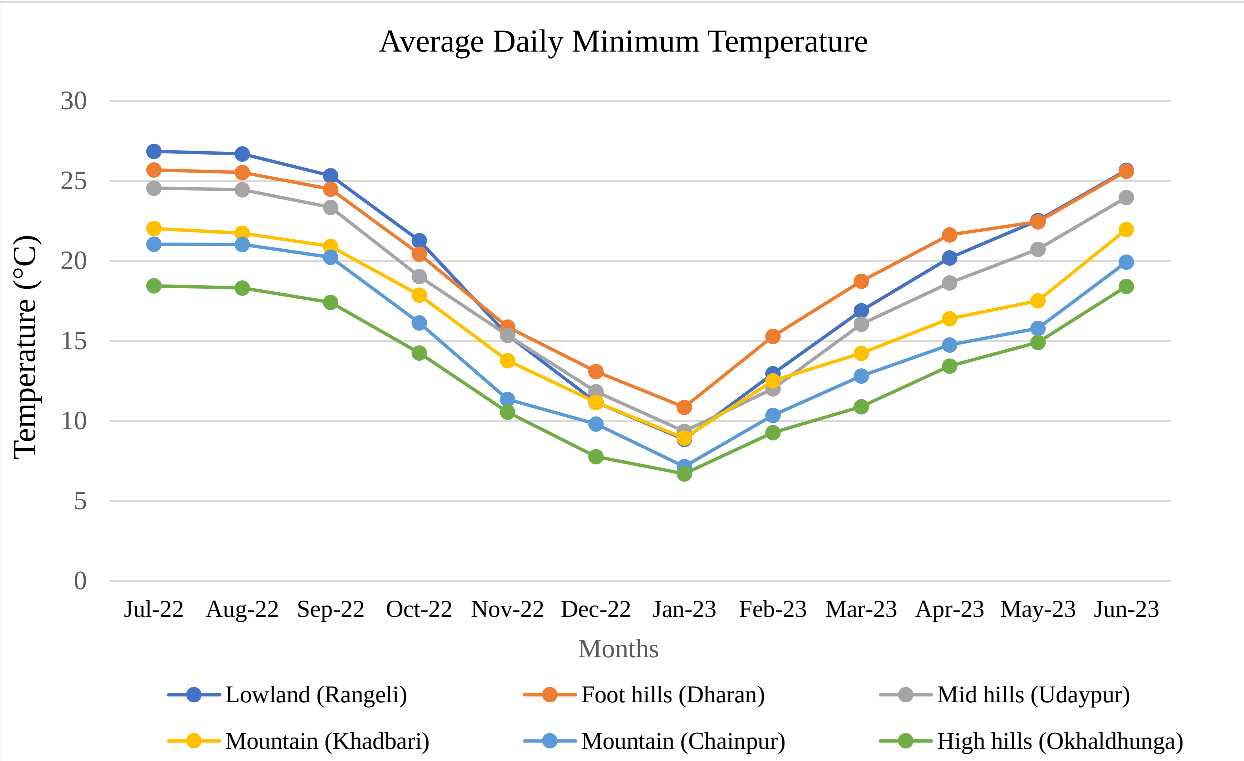

Figure 4. Average daily minimum temperature in survey districts (July 2022- June2023)

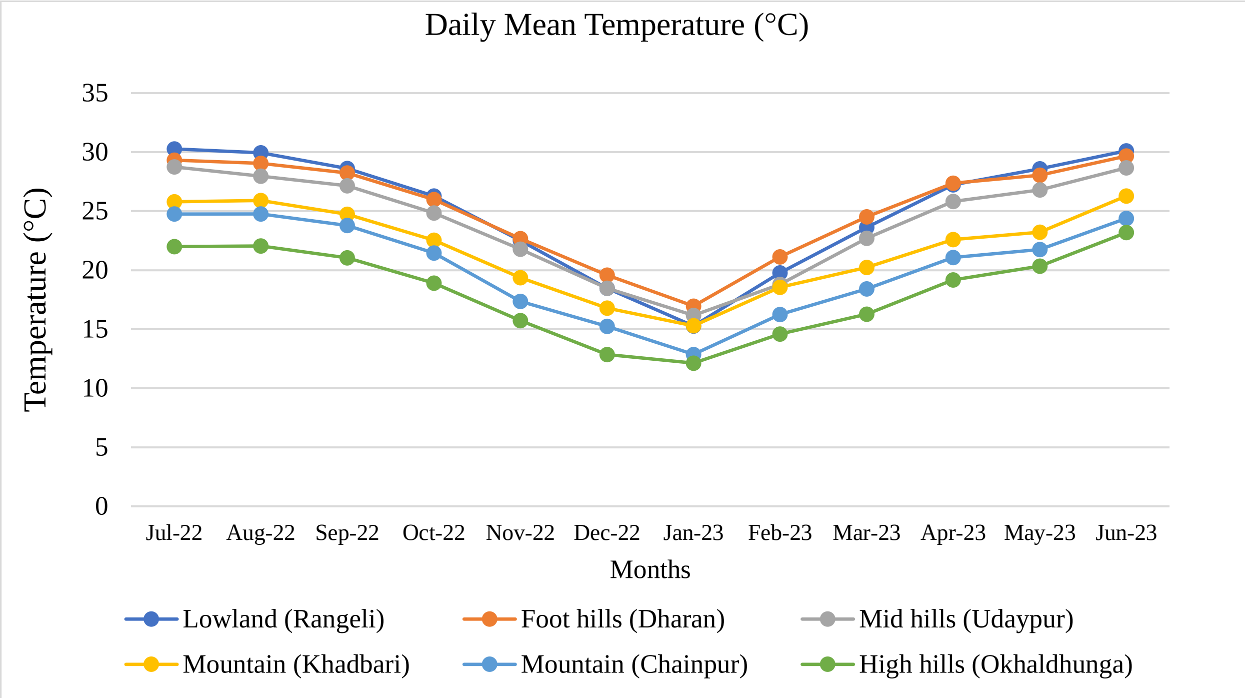

Figure 5. Average daily mean temperature in survey districts (July 2022- June 2023)

Relative humidity

Average daily relative humidity observed in the survey clusters/districts varied from 55.7% in the month of April to 89.1% in the month of September. April month was marked as the driest month and September as the wettest month in terms of moisture present in air. The observed relative humidity varied from 70.2% in lowland (Morang district) to 80.8% in high hills (Okhaldhunga district). Average annual relative humidity was found to be higher in high hills (Okhaldhunga district) compared to all other surveyed districts (Figure 6).

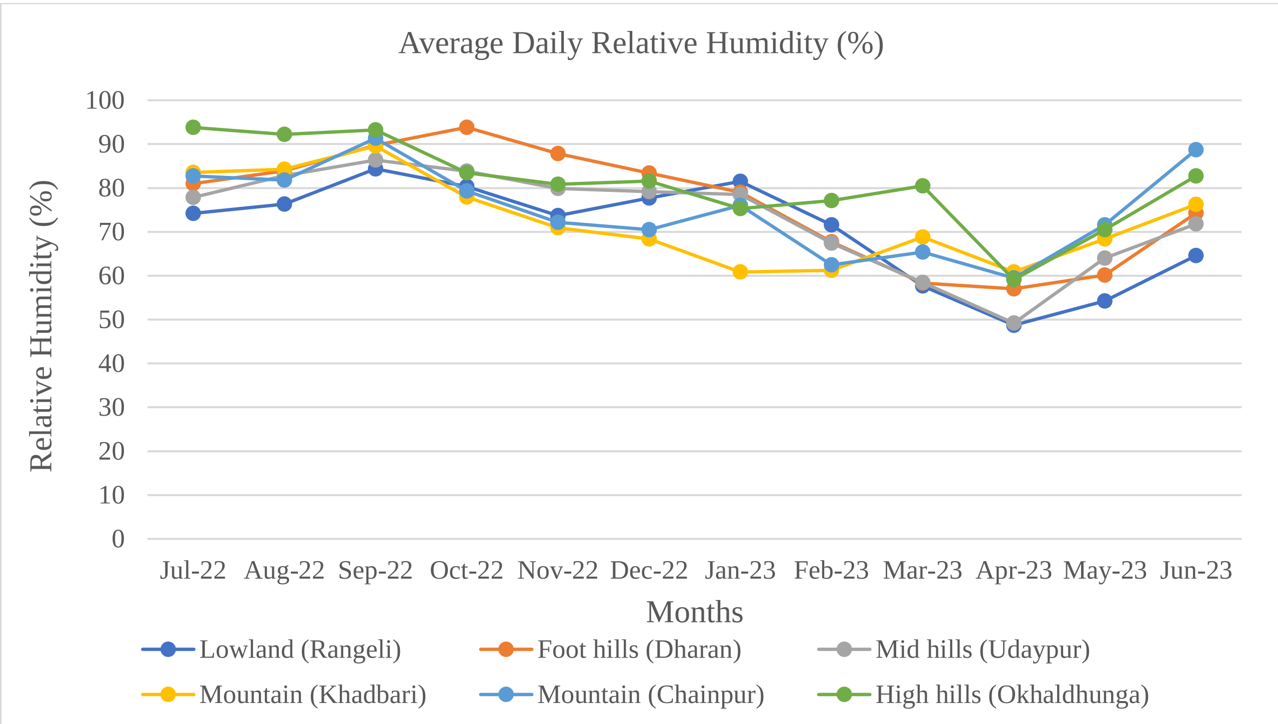

Figure 6. Average daily relative humidity in survey districts (July 2022- June 2023)

Rainfall

It was observed that annual rainfall was different in the three ecological regions; the lowest annual rainfall (1451.6mm) was observed in one of the survey cluster located in lowland (Rangeli, Morang) and highest rainfall (2037.7mm) in the mid hills (Udayapur). Higher average daily rainfall was observed in June to September (Figure 7).

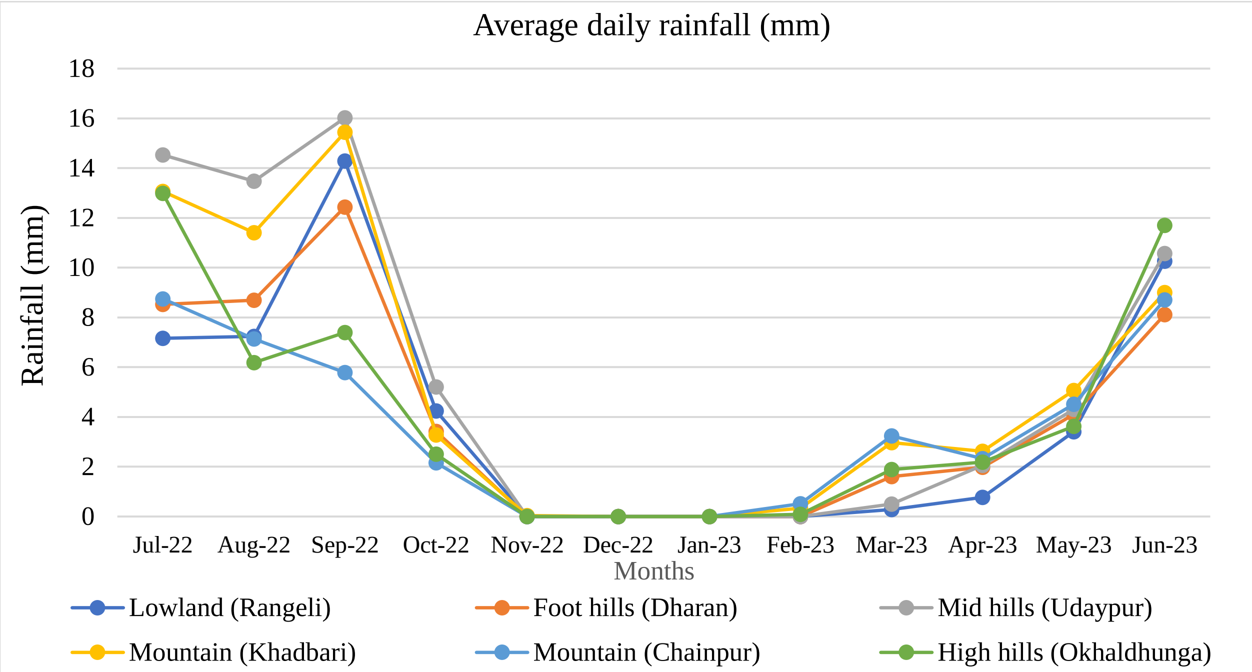

Figure 7. Average daily rainfall (in mm) in survey districts (July 2022- June2023)

7.3 Entomological findings

7.3.1 Abundance and types of vector species

Total number of mosquitoes and sand flies captured was 3,867, of which, vector species comprised of 77.4% (n = 2,994). Altogether six genera with 28 species of mosquitoes and 2 genera with 3 known species of sand flies were captured during the survey. Few specimens (*n* =11) of genus *Phlebotomus* could not be identified up to species level. Variation in the species composition of mosquitoes was evident in surveyed clusters and districts. Diversity index was higher in Okhaldhunga district for all species collected (H' = 1.45) as well as for vector species (H' = 0.57). Species richness for both vector and non-vector species was higher in Udayapur district (S = 20), the same was higher in Okhaldhunga district (S = 5) while only vector species were considered. Pielou’s evenness index illustrated the fact that one or few species were dominant, and others were present with nominal density at the time of collection (Table 3 and 4).

Table 3. Diversity index, evenness index, species richness and abundance of all vector and non-vector species in five districts of Koshi Province, 2023

Districts	**Both vector and non-vector species**				**Vector species only**			
	H'	J	S	A	H'	J	S	A
Morang	0.96	0.36	14	906	0.10	0.09	3	704
Sunsari	1.23	0.49	12	352	0.51	0.37	4	163
Udayapur	0.90	0.30	20	1259	0.09	0.07	4	1021
Okhaldhunga	1.45	0.55	14	395	0.57	0.36	5	254
Sankhuwasabha	0.70	0.23	20	955	0.20	0.14	4	852

Note: H' – Shannon’s diversity index, J – Pielou’s species richness index, S – Species richness, A - Abundance

Table 4. Distribution and abundance of vector and non-vector species in 10 clusters of five survey districts in Koshi province, 2023

Districts	**Morang**		**Sunsari**		**Udayapur**		**Okhaldhunga**		**Sankhuwasabha**		Total, n (%)
Clusters	Pathari Sanischare-7	Rangeli-6	Dharan-8	Dharan-15	Chaudandigadhi-10	Triyuga-6	Manebhanjyang-5	Manebhanjyang-6	Chainpur-10	Khandbari-9	
Ecological region	Lowland	Lowland	Lowland	Lowland	Hills	Hills	Hills	Hills	Mountain	Mountain	
Aedes aegypti	-	-	5	7	-	-	-	-	-	-	12 (0.31)
Aedes albopictus	-	-	1	-	-	-	-	-	-	1	2 (0.05)
Aedes pseudotaeniatus	-	-	-	-	-	-	1	-	-	-	1 (0.03)
Aedes sp.	-	-	-	-	-	-	-	1	-	-	1 (0.03
Anopheles aconitus	-	-	-	-	-	1	-	-	-	-	1 (0.03)
Anopheles annularis	4	1	-	-	-	9	1	-	1	2	18 (0.47)
Anopheles barbirostris	-	-	-	-	-	2	-	-	-	-	2 (0.05)
Anopheles culicifacies	2	2	2	1	22	62	1	1	1	4	98 (2.53)
Anopheles pseudowillmori	-	-	-	-	-	-	12	2	-	-	14 (0.36)
Anopheles stephensi	-	-	5	1	-	1	-	-	-	-	7 (0.18)
Anopheles subpictus	3	66	1	-	-	5	-	-	3	-	78 (2.02)
Anopheles UN1	-	-	-	-	-	1	-	-	-		1 (0.03)
Anopheles vagus	7	27	-	-	2	29	-	-	-	3	68 (1.76)
Anopheles willmori	-	-	-	-	-	-	5	-	-	-	5 (0.13)
Armegeres kesseli	22	-	-	-	6	18	-	-	-	22	68 (1.76)
Armegeres kuchingensis	-	-	-	-	-	1	-	-	-	-	1 (0.03)
Armegeres subalbatus	-	2	3	3	1	-	-	-	-	4	13 (0.34)
Culex bitaeniorhynchus	1	-	-	-	-	3	-	-	-	-	4 (0.10)

Culex fuscocephala	1	-	3	1	-	16	1	-	5	27	54 (1.40)
Culex infula	1	-	-	-	-	-	-	-	-	-	1 (0.03)
Culex mimulus	-	-	-	-	-	1	-	-	-	-	1 (0.03)
Culex quinquefasciatus	256	435	66	75	3	1003	7	10	96	718	2669 (69.02)
Culex sinensis	1	-	-	-	-	-	-	-	-	-	1 (0.03)
Culex tritaeniorhynchus	-	-	-	-	-	2	-	-	-	-	2 (0.05)
Culex vagans	-	-	-	1	-	-	-	-	-	-	1 (0.03)
Culex whitei	-	2	-	-	-	2	-	-	-	-	4 (0.10)
Mansonia uniformis	-	-	-	1	-	2	-	-	-	-	3 (0.08)
*Phlebotomu*s (Adlerius) spp.	-	-	-	-	-	-	2	1	-	-	3 (0.08)
Phlebotomus argentipes	2	6	4	5	2	2	43	174	27	7	272 (7.03)
Phlebotomus major s.l.	-	-	-	-	-	-	29	20	3	1	53 (1.37)
Phlebotomus spp.	-	-	-	-	-	-	7	4	-	-	11 (0.28)
Sergentomyia spp.	42	23	14	153	23	40	18	54	1	29	397 (10.27)
Uranotaenia sp. complex							1				1 (0.03)
Grand Total	**342**	**564**	**104**	**248**	**59**	**1200**	**128**	**267**	**137**	**818**	**3867**

Note: Vector species are highlighted in their respective rows

7.3.2 Distribution of vector species among the districts and altitudinal gradient

The known malaria vectors in Nepal, *Anopheles annularis* were captured from all the surveyed districts except Sunsari located at the altitudes of 98m to >1000m asl (lowlands to highlands). The other vectors for the malaria parasite, *An. pseudowillmori* and *An. willmori* were recorded at around 1200m altitude in Okhaldhunga district only.

The vector of lymphatic filariasis, *Culex quinquefasciatus* is found from 98 to 1274m asl during this survey. Similarly, the vector for Japanese encephalitis, *Culex tritaeniorhynchus*, was found in 632m asl in Udayapur districts in eastern Nepal. The dengue virus vectors, *Aedes aegypti* and *Aedes albopictus* were recorded from 318m in Sunsari district and only *Ae. albopictus* was recorded from 832m asl in Sankhuwasabha district.

Vector for kala-azar, sand fly; *Phlebotomus argentipes* was recorded in 98 to 1274m asl, however, the sand fly abundance was four times higher in surveyed clusters of Okhaldhunga district with altitude >1000m asl. Other suspected vectors for kala-azar, *Ph.* major sensu lato and *Ph. (Adlerius)* sp. were collected from Okhaldhunga district while only the former species was collected from the Sankhuwasabha district at altitudes from 832 to 1011m asl. The details of the vector distribution in the survey districts are given in landscape map (Figure 8 and 9).

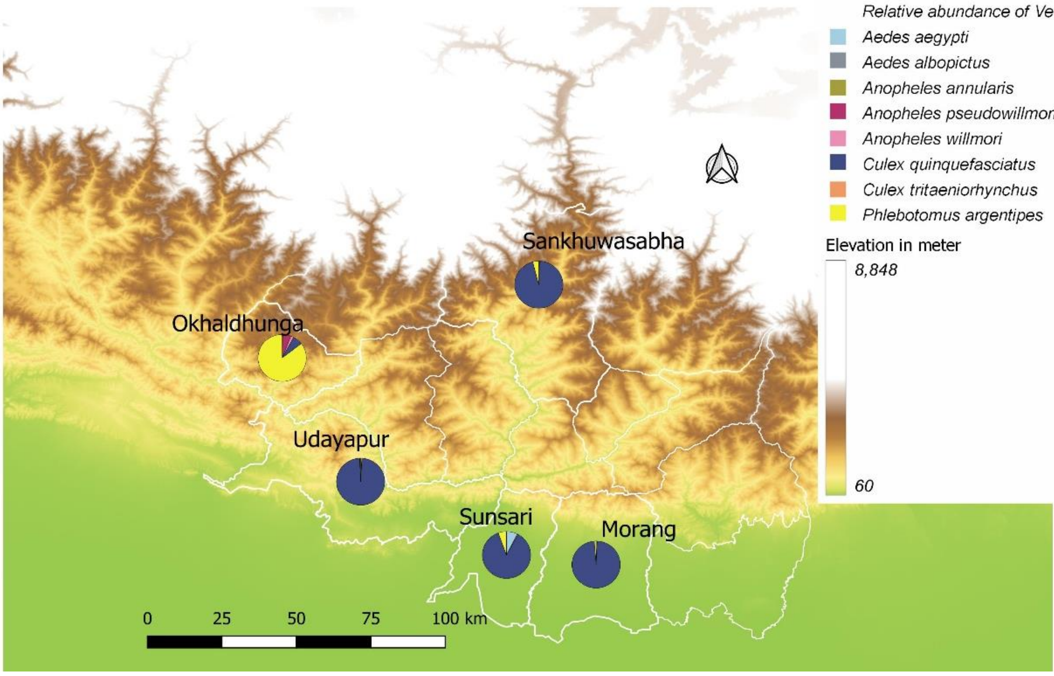

Figure 8. Location where relative abundance (proportion) of the vector species captured in survey districts (Map showing the elevation of the landscape; brown colour- high elevation and green colour- low elevation)

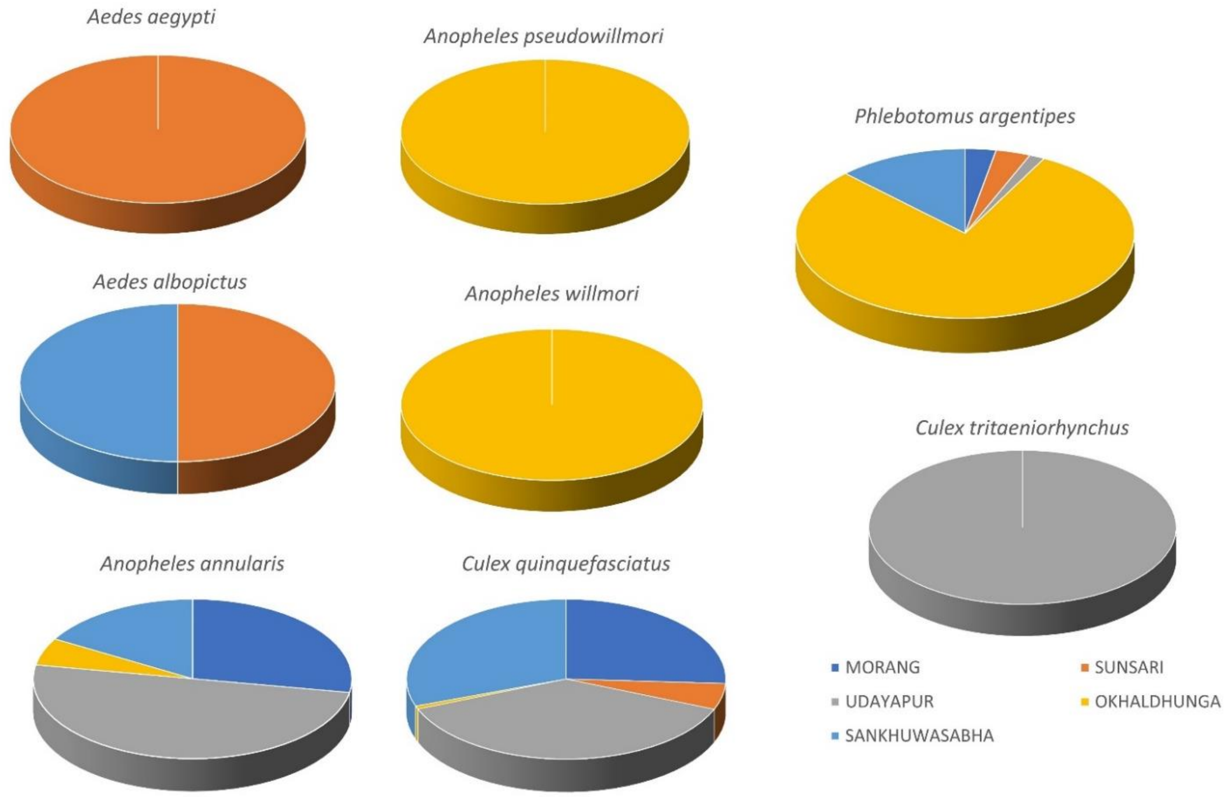

Figure 9. Relative abundance (proportion) of each vector species in survey districts, 2023

7.3.3 Immature stage survey and Stegomyia indices

A. Immature stages other than *Aedes* mosquitoes

In Morang district, three water bodies (River, Ponds and Drain) near the survey clusters were searched for the presence of immature stages of mosquitoes. Water bodies present within the 200 meters of the survey clusters in Udayapur district were surveyed, of which, no immature stages were collected from the river. In Okhaldhunga district, ditches have a high yield of immature stages (>4 per dip). In Sankhuwasabha, only one water body was surveyed which was positive for the larval stage.

B. Immature stages of *Aedes* mosquitoes

In two selected wards of Dharan sub-metropolitan city, the areas had outbreak of dengue in 2019, a total of 434 wet containers in 135 households (including few public places) of 525 population were inspected for the *Aedes* larvae and pupae. Altogether 81 households with 144 wet containers were found to be positive for the immature stages of *Aedes* spp (Table 5). The household index (HI) was found to be 60% (81/135*100), the container index (CI) was 33.18% (144/343*100), Breteau index (BI) was 106.67 (144/135*100). Approximately 443 pupae were collected from the positive containers. The calculated pupae per person was 0.84. These high Stegomyia indices (HI, CI, BI and PPP) indicated the outbreak situation for dengue in the surveyed areas.

Table 5. Type of wet containers searched and their contribution to larval productivity.

Type of wet containers	Searched containers (%)	Positive containers (%)
Bowl	6 (1.38)	6 (4.17)
Bucket	66 (15.21)	11 (7.64)
Cement tank	2 (0.46)	2 (1.39)
Ceramic earthen jar	5 (1.15)	2 (1.39)
Coconut shell	2 (0.46)	0 (0.00)
Discarded bottles	15 (3.46)	5 (3.47)
Discarded plastic container	15 (3.46)	10 (6.94)
Discarded Tins	6 (1.38)	0 (0.00)
Ditch	2 (0.46)	1 (0.69)
Drum	242 (55.76)	63 (43.75)
Flower vase	48 (11.06)	27 (18.75)
Gallon	2 (0.46)	1 (0.69)
Mud pot	2 (0.46)	1 (0.69)
Plastic jar	7 (1.61)	5 (3.47)
Tyre	14 (3.23)	10 (6.94)

7.4 Association between vector abundance and geo-ecological and climatic factors

Two vector species; *Cu. quinqfasciatus* and *P. argentipes* were considered for the regression analysis as they were present in the higher number (n = 2669, 89.14% and (n = 272, 9.01%) as compared to other vector species (n = 53, 1.77%). Association of the geo-ecological and climatic factors are represented separately.

For *Cu. quinquefasciatus*: The effect of topography had a significant effect on the mean abundance of *Cu. quinquefasciatus*. Higher collections were made per household in hills (IRR = 1.23, CI at 95% = 0.53 – 2.87) and mountain (IRR = 1.96, CI at 95% = 0.73 – 5.91) as compared to lowland. The result also indicated the existence of higher density of this vector in higher altitudes. CDC light trap was found to be excellent method of collection compared to aspirator (IRR = 0.05, CI at 95% = 0.03 – 0.08) and BG sentinel traps (IRR = 0.06, CI at 95% = 0.01 – 0.60). Vector density was found to be very low in cattle sheds, mixed dwellings and outdoors as compared to human dwellings (Table 6).

Considering the household structures, houses with tiled roofs, mud walls and cemented floor had more vector density than other types of roof, wall or floor (Table 6). Vector density per household was found to be less in well ventilated rooms (IRR = 0.36, CI at 95% = 0.14 – 1.13) as compared to houses without proper ventilation. Other ecological factors like presence of goats, pigs, agricultural field, mixed orchard with variety of tropical and subtropical plants present nearby the household, presence of river, ponds and drains have increasing effect Cu. quinquefasciatus density per household (Table 6).

A weak but significant correlation with the *Cu. quinquefasciatus* density was observed with the mean temperature ($r = 0.38$, $p<0.001$) and rainfall ($r = 0.11$, $p<0.001$) while the same was inversely proportional to the mean relative humidity of April and May ($r = -0.32$, $p < 0.001$) and no correlation with rainfall ($r = 0.11$, $p = 0.27$). When these climatic factors were fitted in the model, the mean temperature of April and May had an increasing effect on the vector density (IRR = 1.19, CI at 95% = 1.01 – 1.40). Rainfall had an overall negligible increasing effect but while analysed at ecological region, the model demonstrated increasing effect in hills and mountain but decreasing in the lowland. Another climatic variable, relative humidity had decreasing effect on the density of this vector per household in all ecological regions (Figure 10 A, B, C).

For *P. argentipes*: A significant effect of topography is also seen with *P. argentipes* density. Almost 13 and four times more collections per household were observed in hilly and mountainous districts as compared to lowlands and this result indicated a well-established vector density in higher altitudes. CDC light traps were found to be a highly efficient method of collection as compared to other methods. Mixed dwellings were found be highly productive in vector density (IRR = 12.18, CI at 95% = 1.87 – 789.54) compared to other sites of collection. Houses with thatched roofs, cemented walls, earthen floors, and poor or no ventilation showed an increasing effect on the vector density. Other ecological factors like the presence of cattle, goats, agricultural fields, vegetable fields, rivers, ponds, and ditches showed increasing effects on *P. argentipes* density per household (Table 6).

Phlebotomus argentipes density showed negative correlation with temperature ($r = -0.51$, $p<0.001$). A weak but significant correlation of vector density with relative humidity was observed ($r = 0.50$, $p<0.001$) and negative insignificant relation with rainfall ($r = -0.10$, $p = 0.32$). While fitted in the model with climatic data from April and May, the mean relative humidity showed an increasing effect on vector density (IRR = 1.29, CI at 95% = 1.20 – 1.41), the mean temperature had a decreasing effect and the cumulative rainfall showed no effect on the vector density (Table 6, Figure 10D, E, F).

Table 6. Association between geo-ecological and climatic factors with *Cu. quinquefasciatus* and *P. argentipes* density.

Explanatory variables		**IRR (CI at 95%) for** *Culex quinquefasciatus*	**IRR (CI at 95%) for** *Phlebotomus argentipes*
General			
Ecological region (ref: Lowland)	Intercept	20.80 (12.11 – 40.07)	0.43 (0.20 – 0.92)
	Hills	**1.23** (0.53 – 2.87)	**13.00** (5.06 – 34.04)
	Mountain	**1.96** (0.73 – 5.91)	**4.00** (1.31 – 13.38)
Method of collection (ref: Light trap)	Intercept	12.55 (9.07 – 18.04)	1.06 (0.67 – 1.79)
	Aspirator	0.05 (0.03 – 0.08)	0.18 (0.09 – 0.34)
	BG sentinel trap	0.06 (0.01 – 0.60)	0.63 (0.09 – 19.66)
Collection sites (ref: Human dwelling)	Intercept	6.72 (5.05 – 8.95)	0.43 (0.30 – 0.65)
	Cattle shed	0.04 (0.02 – 0.09)	**1.34** (0.59 – 3.44)
	Mixed dwelling	00 (00 – 00)	**12.18** (1.87 – 789.54)
	Outdoor	0.12 (0.02 – 0.89)	**1.55** (0.21 – 73.47)
Household structure			
Type of roof (ref: Tin)	Intercept	28.42 (19.20 – 44.57)	2.08 (1.26 – 3.41)
	Cemented	0.13 (0.02 – 3.41)	0
	Thatched	0.61 (0.25 – 1.81)	**2.94** (0.96 – 9.00)
	Tiles	4.57 (0.42 – 11017.67)	0
Type of floor (ref: Earthen)	Intercept	25.61 (16.68 – 42.16)	2.84 (1.68 – 5.25)
	Cement	1.13 (0.52 – 2.64)	0.88 (0.34 – 2.50)
Type of wall (ref: Mud)	Intercept	31.63 (20.84 – 51.28)	2.44 (1.45 – 4.48)
	Cemented	0.62 (0.27 – 1.61)	**1.57** (0.57 – 5.17)
	Tin	0.24 (0.03 – 19.27)	0.41 (0.02 – 164.73)
	Unplastered	0.17 (0.04 – 1.31)	0.82 (0.16 – 11.47)
Ventilation present (ref: No)	Intercept	30.14 (20.59 – 46.59)	2.99 (1.87 – 5.13)
	Yes	0.33 (0.13 – 1.00)	0.47 (0.15 – 1.96)
Ecological variables factors			
Cattle present (ref: No)	Intercept	31.23 (19.95 – 52.82)	0.98 (0.57 – 1.80)
	Yes	0.64 (0.30 – 1.41)	**5.42** (2.34 – 13.05)
Goat present (ref: No)	Intercept	22.91 (13.69 – 42.52)	0.40 (0.20 – 0.83)
	Yes	**1.30** (0.60 – 2.77)	**11.55** (4.83 – 27.79)

Pigs present (ref: No)	Intercept	2.67 (1.60 – 4.88)	2.67 (1.60 – 4.88)
	Yes	**1.06** (0.40 – 3.15)	1.06 (0.40 – 3.15)
Cow dung near the house (ref: No)	Intercept	33.88 (20.74 – 60.79)	0.78 (0.42 – 1.54)
	Yes	0.58 (0.27 – 1.24)	5.92 (2.49 – 14.06)
Agricultural field present (ref: No)	Intercept	12.53 (7.04 – 25.38)	2.56 (1.25 – 6.29)
	Yes	**2.71** (1.19 – 5.78)	**1.10** (0.39 – 2.83)
Vegetable field present (ref: No)	Intercept	31.25 (17.69 – 62.87)	1.42 (0.70 – 3.35)
	Yes	0.77 (0.34 – 1.66)	**2.44** (0.89 – 6.21)
Mixed orchard present (ref: No)	Intercept	23.21 (9.75 – 77.60)	3.36 (1.18 – 15.14)
	Yes	**1.17** (0.33 – 3.12)	0.78 (0.16 – 2.24)
River present (ref: No)	Intercept	25.03 (14.34 – 49.41)	1.5 (0.75 – 3.46)
	Yes	**1.11** (0.49 – 2.37)	**2.31** (0.86 – 5.85)
Ponds present (ref: No)	Intercept	16.82 (11.33 – 26.45)	2.69 (1.63 – 4.82)
	Yes	**3.26** (1.49 – 7.93)	**1.04** (0.39 – 3.32)
Drains present (ref: No)	Intercept	18.02 (11.72 – 29.68)	3.94 (2.44 – 6.85)
	Yes	**2.34** (1.10 – 5.23)	0.14 (0.06 – 0.37)
Ditches present (ref: No)	Intercept	26.75 (17.26 – 44.59)	2.55 (1.49 – 4.79)
	Yes	0.99 (0.46 – 2.27)	**1.19** (0.47 – 3.28)
Climatic factors			
Temperature	Intercept	0.32 (0.01 – 19.11)	33153(3638.95 – 359832.80)
	Mean temp (April and May)	**1.19** (1.01 – 1.40)	0.66 (0.59 – 0.72)
Relative humidity	Intercept	595.78 (8.30 – 52598.68)	0
	Mean RH (April and May)	0.95 (0.88 – 1.02)	**1.29** (1.20 – 1.41)
Rainfall	Intercept	12.36 (2.36 – 77.42)	3.86 (0.03 – 472.73)
	Cumulative Rainfall (April and May)	1.00 (0.99 – 1.01)	1.00 (0.97 – 1.03)

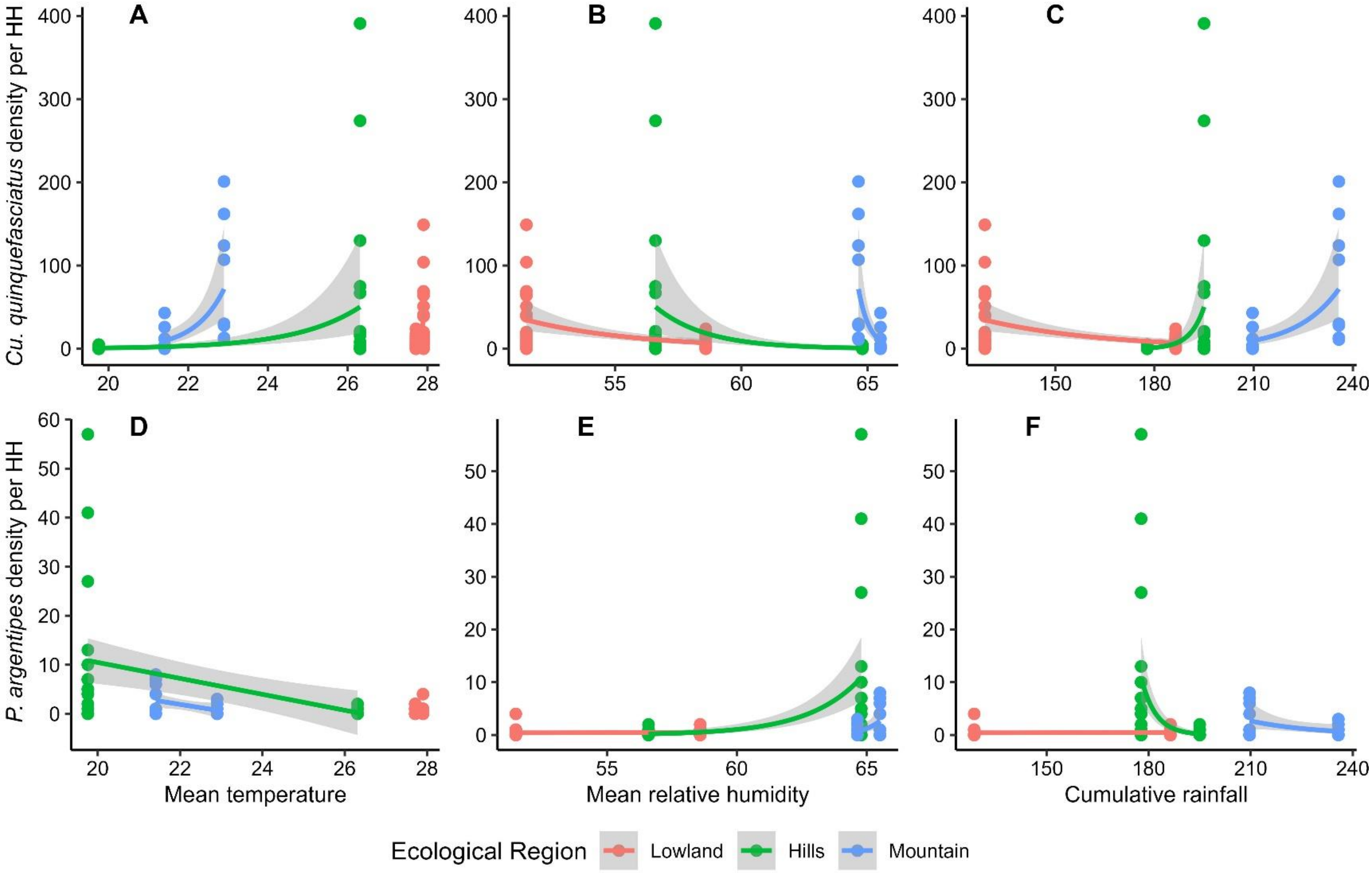

Figure 10. Scattered plots and regression lines showing effects of climatic variables on vector density in three ecological regions. Panels A, B and C show effects of climatic variables on *Culex quinquefasciatus* density per HH (household) and panels D, E and F show effects on *Phlebotomus argentipes* density per HH. Dots represent the data points, line represents the generalized regression line with negative binomial distribution and shaded area indicates the standard error of the regression line.

7.5 Association between vector abundance and presence of vector-borne diseases

Incidence rates of LF and VL were analysed with their respective vectors and gradient maps were constructed. The high disease incidence for LF was well coincided with the high Cu. quinquefasciatus abundance in Udaypur district (Figure 11). The LF incidence rate was not available in national line list for Morang and Sankhuwasabha districts. Similar pattern was seen for VL as well, the high incidence rate of the disease was in the same district where large number of *P. argentipes* were collected (Figure 12).

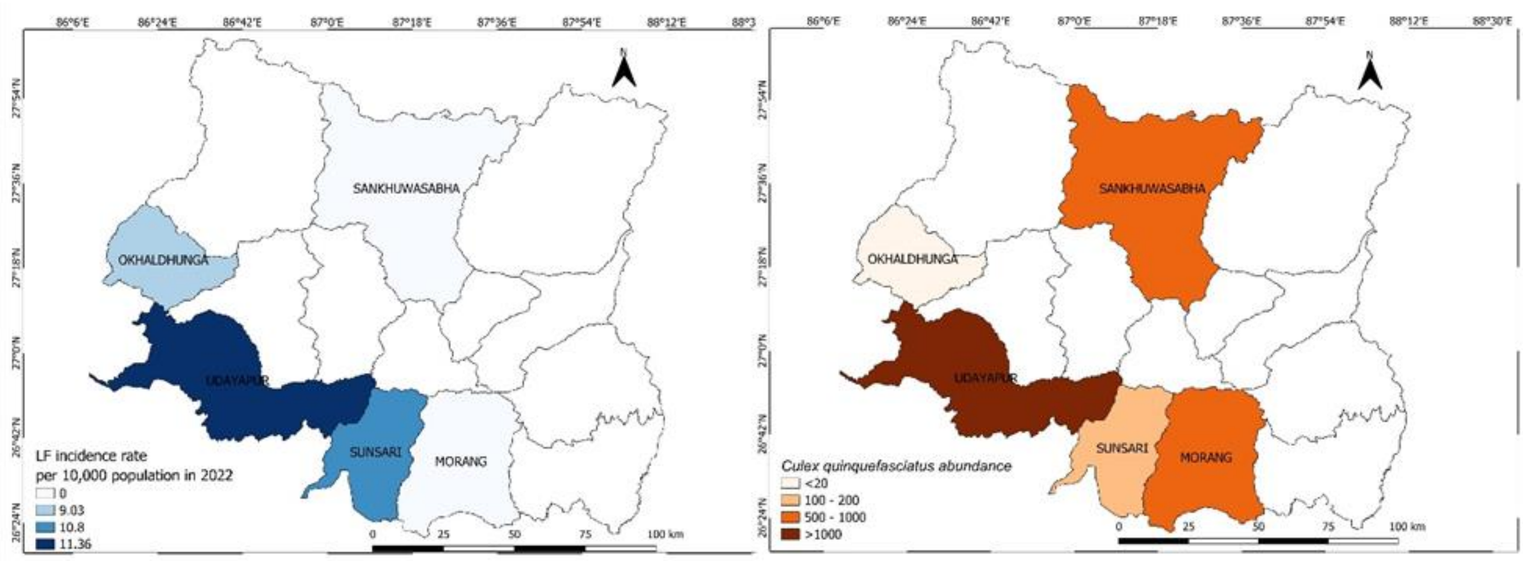

Figure 11. Lymphatic filariasis incidence rates in 2022 and *Culex quinquefasciatus* abundance during the survey in five study districts in Koshi province.

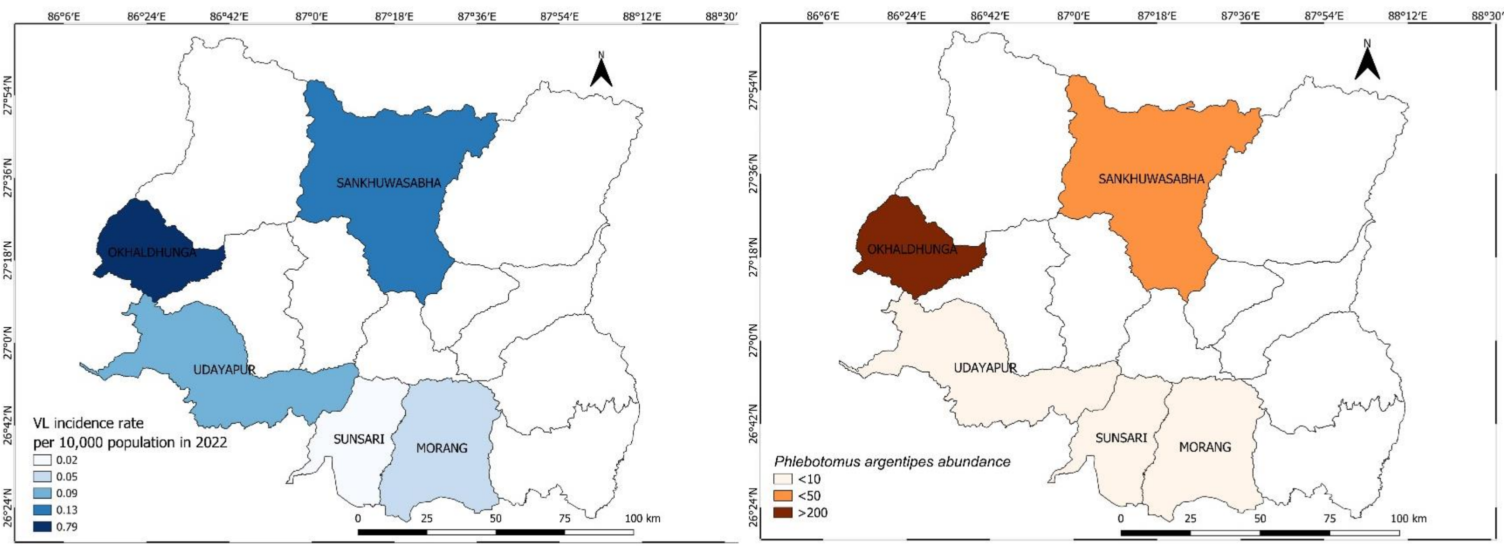

Figure 12. Visceral leishmaniasis incidence rates in 2022 and *Phlebotomus argentipes* abundance during the survey in five study districts in Koshi province.

When fitted in the model, the disease incidence for LF was found not to be associated with the vector density (might be due to high incidence of LF - 9.03 per 10,000 population – with very less number of vector collection - only 17 *Cu. quinquefasciatus*). However, higher density of *P. argentipes* had increasing effect on VL incidence rate (IRR = 1.04, CI at 95% = 1.01 – 1.06) at district level (Table 7).

Table 7. Effect of the vector density on the incidence rates of major VBDs at the district level in Koshi province.

Explanatory variables		**IRR (CI at 95%) for** Lymphatic filariasis incidence rate	**IRR (CI at 95%) for** Visceral leishmaniasis incidence rate
Culex quinquefasciatus	Intercept	0.65 (6.01 – 7.13)	-
	Density at household level	1.00 (1.00 – 1.00)	-
Phlebotomus argentipes	Intercept	-	0.18 (0.11 – 0.28)
	Density at household level	-	**1.04** (1.01 – 1.06)

8. Conclusions

This cross-sectional vector survey provides baseline information on the distribution of vectors of major VBDs in different geo-ecological and climate variances in Koshi Province of Nepal. There was not only evidence of the presence of vectors in all geo-ecological zones but the vector populations were well established there. The two abundant vector species; *Cu. quinquefasciatus* and *P. argentipes* were indiscriminately present in lowlands to highlands (hills and mountains). The presence of vectors in higher altitudes could be attributed to changes in climatic variables which are suitable for the survival, distribution and growth of vector population. Integrated vector surveillance is preferred over disease specific vector surveillance for better use of fund/resources and to generate comprehensive data on vectors' diversity. In addition, these findings alert to the control program for regular monitoring, strengthening the existing surveillance and timely control interventions in these previously disease-free and environmentally suitability areas for VBDs transmission.

9. Recommendations

The following recommendations are made for further strengthening of vector surveillance and management of VBDs through vector control:

1. A one-time integrated vector survey in a very limited study area does not suffice for the generalization of diversity, distribution, and bionomics of the vectors, hence year-round surveillance of vectors in wider geographical regions is highly recommended.
2. Sustainable integrated vector surveillance in selected sentinel sites present in various geo-ecological zones is necessary for evidence-based decision-making for the implementation of effective vector control methods.
3. The habitat reductions and awareness to the community can be performed parallel for dengue vector surveillance. The practice should be routinely performed during the non-transmission season as well.
4. Xenomonitoring of the pathogens in the vector populations can be investigated to assess the risk of pathogen transmission in a human population living in endemic or non-endemic areas.
5. In case of outbreaks of a particular VBDs, targeted vector surveillance should be conducted to provide evidence for prompt action on the vector control and management.

10. Acknowledgements

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11. Annexes

Annex – 1: Adult vector survey data collection form

Household Identification

1. Province:________2. District:___________3. Municipality (Urban/Rural):________________

4. Ward no.:/__/ 5. Village/Cluster:_________________6. GPS location: Latitude_____________Longitude______________ 7. Elevation_________8. Weather condition:___________________ 9. Collection day/night: /____/

10. Name of household_______________________________11. Location description:_______________________

11. Method of collection: (i) CDC light traps ☐ [27] BG sentinel traps ☐ (iii) Cattle baited ☐

(iv) Manual aspirator (v) Human baited double net traps ☐ (vi) Human landing catches ☐

Genus	Species	Sex and abdominal status					Total
		Number of Males	Number of Females				
			Unfed	Fed	Semi-gravid	Gravid	

Code of collector/s:___________________________Date of collection (yy/mm/dd):/________/____/____/

Entomologist:________________________________Date of speciation (yy/mm/dd):/________/____/____

Annex - 2: Household survey form (Base-line observation checklist)

1. Household Identification

1.1 District ____________________VDC_____________________Village_________________

1.2 House No. [____]

1.3 GPS location done (Yes/No): [____]

1.4 Latitude: N____^{0}_____._______;Longitude: E____^{0}_____._______Elevation:____,____m (asl)

1.5 Name of the Head of the household_______________________________

1.6 Contact: mobile phone:____

1.7 Number of family members:_[   ]

2. Description of Housing and living conditions

2.1 Type of housing:

2.1.1. Number of separate buildings within the compound [____]

2.1.2. Number of 2 story-buildings [____]

Drawing:

2.2 Kitchen facilities:

2.2.1. Type: 1= within a common room, 2= separate room, 3= in open space) [___]

2.2.2 Location: 1= Ground floor, 2 = upper floor [___]

2.3 Living or Dining room

2.3.1. Type: 1= within a common room, 2= separate room, 3= in open space) [___]

2.3.2 Location: 1= Ground floor, 2 = upper floor [___]

2.4 Sleeping facilities:

2.4.1. Type: 1= within a common room, 2= separate room [___]

2.4.1. If separate: number of sleeping rooms [___]

2.4.2 Location: 1= Ground floor, 2 = upper floor [___]

2.5 Type of room/building used for sleeping:

Title	Description	Mixed dwelling/Cattle Shed	Sleeping rooms		
			room 1	room 2	room 3
LTused in					
Used by:	1= adults 2= children 3= mixed				
Persons	Persons/bedroom				
Bedding	Number of beds				
	Number of mats (= on the floor)				
Roof type	1= Thatched 2= Tin 3= Tiles 4.=Concrete/Cemented 5= If others (Mention)	|__| …………	|__| …………	|__| …………	|__| …………
Floor type	1= Mud/earthen 2= Cement 3= Mud and cement 4= If others (Mention)	|__| …………	|__| …………	|__| …………	|__| …………
Wall type	1= Mud/earthen 2= Cemented/plastered 3= Tin 4=Unplastered wood/bamboo 5= Unplastered brick 6= If others (Mention)	|__| …………	|__| …………	|__| …………	|__| …………
Cracks in wall	1= Inside the wall 2= Outside the wall 3= Inside and outside both 4=No cracks		|__| …………	|__| …………	|__| …………
Door	**Type:** 1= real door 2= curtain				
Window	**Type:** 1= none; 2= open (ventilated/glass tiles) 3=				

	closed (bamboo/wooden/full glass)				
	Mosquito screen: 1= yes 2= no				
Remarks					

2.6 Does the house have a veranda where people sleep during the hot season? (1= Yes, 2= No) [_____]

2.7. Other constructions present <u>inside</u> the house compound

2.7.1. Cattle sheds: (1= Yes, 2= No) [_____]

2.7.2. Storage rooms: (1= Yes, 2= No) [_____]

2.7.3. Latrine: (1= Yes, 2= No) [_____]

Latrine type:

2.8. Other constructions <u>outside</u> of the house compound

2.8.1. Similar human dwellings: (1= Yes, 2= No) [_____]

2.8.2. Mixed dwelling: (1= Yes, 2= No) [_____]

2.8.3. Cattle sheds: (1= Yes, 2= No) [_____]

3. Surrounding environment of the house

3.1 Proximity to/of domestic animals (*)

(*) even if these animals are not the property of the household

3.1.1. Are there any domestic animals kept near or inside the house? (1= Yes, 2= No) [_____]

Description	Number	Inside (1 = Day, 2 = Night, 3 = Both)	Outside (1 = Day, 2 = Night, 3 = Both)	Distance from the main house (ft/m)
1.Cow				
2. Buffaloes				
3. Goats				
4. Pigs				

5. Dogs				
6. Chicken				
7. Ducks				
8. Birds				
9. Others………				

3.2 Presence of cow dung nearby the house: (if Yes = 1, No = 2) [_____]

3.3 Type of water bodies present nearby the household

Description	**Status of water logging (1= throughout the year, 2= seasonal); specify the season/s**	**Distance from the house (ft/m)**
1. River		
2. Ponds		
3. Canals		
4. Drains		
5. Ditches		
6. If other, specify…………….		

3.4 Type of vegetation present in the surroundings

Description	**Yes**	**No**	**Type of plants grown (Season)**
1. Agricultural field			e.g Paddy (Rainy), Wheat (Winter), Hemp (spring), etc. Name the crop: ___________________________________
2. Vegetable field			e.g. Cauliflower, brinjal, potato (winter),Okra, cucumber (summer), etc. Name the vegetable ___________________________________
3. Mixed Orchard			e.g. Bamboo+ Litchi + Mango (perennial) Name the mixed plantation ___________________________________
4. Specific Orchard			e.g. Bamboo (perennial) or Mango(perennial), etc. Name the orchard ______________________________

5. If others, specify			

4. Socio-economic status of the family

4.1 Family assets

Description	**Quantity**	**Description**	**Quantity**
1. Agricultural land		13. Van/ Riksha	
2. Motorcycles		14. Power Tiller	
3. Television sets		15. Thresher Machine	
4. Mobile sets		16. Pumping set	
5. Radio		17. Boat	
6. Fans		18. Almirah	
7. Watches (wall, table, wrist)		19. Tables	
8. Mattresses/Blankets		20. Chairs	
9. Bed/cots		21. Sewing Machine	
10. Bed nets		22. Fish farm	
11. Bicycles		23. Poultry farm	
12. Rice processing mill		24. Others, specify	

4. Others

5.1 Was this house sprayed with IRS in last 6 months? (1= Yes, 2= No) [____]

5.2 Have you ever heard about the following vector borne diseases?

5.2.1 Malaria (1= Yes, 2= No) [____]

5.2.2 Kala-azar (1= Yes, 2= No) [____]

5.2.3 Lymphatic filariasis (1= Yes, 2= No) [____]

5.2.4 Japanese encephalitis (1= Yes, 2= No) [____]

5.2.5 Dengue (1= Yes, 2= No) [____]

5.2.6 Chikungunya/Zika (1= Yes, 2= No) [____]

Annex - 3: Larval/pupal survey data collection form

1. Province:_______2. District:___________3. Municipality (Urban/Rural):_________4. Ward no.:/__/

5. Village/Cluster:_______________6. GPS location: Latitude__________Longitude__________

7. Elevation________ 8. Weather condition:__________9. Date: /________/__/__/

10. Code of collectors/inspectors:__________

Time of collection	Larval habitat	Characterization of the breeding site											Sampling description			
		Type (1=permanent, 2=semi-permanent, 3=temporary)	Origin of water (1=rain, 2=river,	Light Index (1=shaded, 2=partially shaded,	Water movement (1= stagnant, 2= slow,	Water condition (1=clear,	pH of water	Temperature (°C)	Presence of vegetation (1=emergent,	Presence of algae (1=green algae,	Presence of other organisms	House distance (1=>2-5km, 2=<2km,	Number of dips	Number of +ve dips	Number of larvae	Number of pupae
		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O

Annex - 4: *Aedes* sp. larval/pupal survey form for households and public spaces

1. Province:_______________2. District:_______________3. Municipality (Urban/Rural):_____________________ 4. Ward no.:/____/

5. Village/Cluster:_____________________ 6. Location/HH Name:____________________________

7. If HH, Number of people living in the house:/__/__/ 8. If Public space, space code*:/__/__/ 9. Date of collection:/______/__/__/

10. GPS location: Latitude________________Longitude________________11. Elevation_______ 12. Code of collectors/inspectors:_______

		Category A: Water storage containers that are used						Category B: Water storage containers that are not used									
	INDEX	Drum/Barrel	Cement/steel tank	Ceramic/earthen/fiber jar	Bucket	Other: specify…………	Other: specify……………	Ceramic jar	Bowl (Toilet use, fish bowl. Ant trap,	Flower vase	Tyre	Coconut Shell	Discarded tins, bottles, plastic	Natural breeding habitat e.g, plant axil	Other: specify…………	Other: specify…………	Other: specify…………
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Each water container has one row to be filled (containers with water only)	1																
	2																
	3																
	4																
	5																
	6																

Details of each container

										Larval density	Pupae count			
INDEX	Category	Water volume	Type of water (1=tap/well, 2=rain, 3=other)	Under vegetation (1=fully, 2=partially, 3=NA)	Container location (1=inside, 2=outside)	Usage during the past 7 days (1=yes, 2=no)	Shade (1=fully, 2=partially, 3=NA)	Intervention applied (1=yes, 2=no)	Container cover (1=total, 2=partial, 3=no)	0=no larvae, 1=<10, 2=10-50, 3=>50	Absolute number (small container)	Sample/collection factor	Estimated pupal count (large	Remarks
		A	B	C	D	E	F	G	H	I	J	K	L	M
1														
2														
3														
4														
5														
6														

	7																		7														
	8																		8														
	9																		9														
Total																																	

**1= Public street/pathway; 2=Green areas for leisure; 3=Abandoned areas, dumping ground; 4=Public building (school, hospital); 5=Religious buildings; 6=Private corporate houses*

Figure 13. Pictures during field collections and in the lab

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