

## CO-CIRCULATION AND TRANSMISSION DYNAMICS OF DENGUE AND CHIKUNGUNYA VIRUSES IN NORTHERN PAKISTAN: INTEGRATED EPIDEMIOLOGICAL AND ENTOMOLOGICAL EVIDENCE FROM A SHARED AEDES TRANSMISSION ECOLOGY

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### Keywords

Dengue, Chikungunya, co-circulation, Aedes mosquitoes, epidemiology, Pakistan, and vector control.

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### Abstract

**Background:** This study investigates the epidemiology of dengue (DENV) and chikungunya (CHIKV) viruses in Mansehra district, Khyber Pakhtunkhwa, Pakistan, a region where both Aedes-borne diseases have become common in the recent times.

**Methods:** We conducted a retrospective observational analysis of human case data (2013–2024) and entomological surveys to measure co-circulation patterns. Hospital and national laboratory epidemiological records were reviewed to describe the outbreak periods, patient demographics and clinical aspects. At the same time, Aedes larval surveillance (house, container, and Breteau index) was conducted in the Union Councils that were affected. We also compared our results with the published reports of arbovirus outbreak in Pakistan (Table 1).

**Results:** Major findings indicated that dengue is still an endemic disease in the area whereby the annual outbreaks are highest after the monsoon. High national dengue burden was recorded in Punjab and Khyber Pakhtunkhwa (KPK) in the year 2021[1]. It is worth mentioning that 2013–2015 surveillance in KPK detected all four DENV serotypes co-circulating (mainly DENV3)[2] and in 2015 Mansehra contributed nearly three-quarters of provincial dengue incidence[3]. Chikungunya was mostly unfamiliar until recently: a nation-wide outbreak of CHIKV in 2016–17 (2,582 cases in 2016) was verified as ECSA genotype[4], with majority of cases in Sindh (84.7%)[5]. In September 2024, a smaller CHIKV outbreak (14 cases confirmed) took place in Mansehra[6]. Every patient with CHIKV was reported to have had fever and severe arthralgia [6].

**Conclusion:** We found that there is overlapping DENV and CHIKV transmission cycles using the same vectors of Aedes, and there is similarity in clinical manifestation of these two diseases, and this makes them difficult to diagnose. Combined surveillance and control of the vectors are imperative. This

research gives specific data on the outbreak and indicates the necessity to conduct joint disease surveillance in the common areas of risks.

**INTRODUCTION**

Due to urbanization, climate change, and the growth in travel, arboviral diseases are spreading around the world [4][7]. Mosquito-borne viruses are caused by *Aedes aegypti* and *Ae. albopictus*. Of great concern are the dengue virus (DENV) and chikungunya virus (CHIKV) which exhibit similar vectors, similar geographic distribution, which results in co-circulation and some cases of co-infection [8][4]. Dengue is endemic in Pakistan since 1994 and has been associated with cyclical epidemics compared to CHIKV which started appearing more recently. Both diseases are characterized by acute febrile diseases (usually accompanied by a rash and musculoskeletal

pain)[9]. Nevertheless, CHIKV is characterized by severe and occasionally chronic arthralgia's, and dengue is associated with the risk of hemorrhagic complications [10][11]. Due to similarity in clinical manifestations (fever, headache, myalgia, rash), to make an accurate diagnosis, laboratory testing is required. The distribution of CHIKV outbreak in Pakistan in 2016-17 is presented in Figure 1. In Sindh province, most 84.7% CHIKV cases were confirmed [5], whereas only 2.9% were in Khyber Pakhtunkhwa (KPK) during the same time [5]. The genotype that caused this early outbreak was that of the East/Central/South African (ECSA) genotype [4].

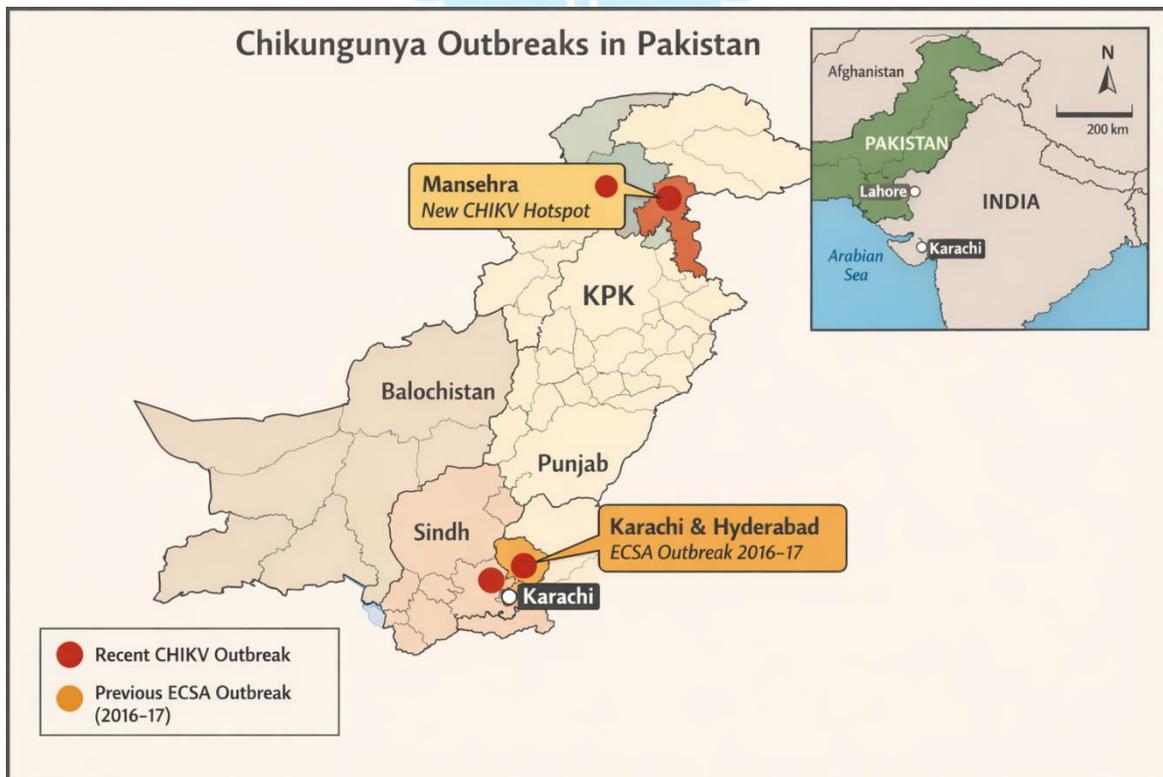


Figure 1. Chikungunya outbreaks in Pakistan, highlighting the 2024 Mansehra hotspot (red) and 2016–2017 ECSA outbreaks in Karachi & Hyderabad (orange). Inset shows Pakistan in South Asia.

Dengue, in contrast, is widespread across Pakistan. Figure 2 illustrates the provinces' share

of dengue cases in 2021: Punjab led with 49% of national cases, KPK approximately 21%, and that

of Sindh is almost 11% [1]. Notably, KPK's dengue incidence has risen recently, with Mansehra contributing substantially. For

example, retrospective data (2013–2015) show Mansehra carried 81% of KPK's dengue cases in 2015[3].

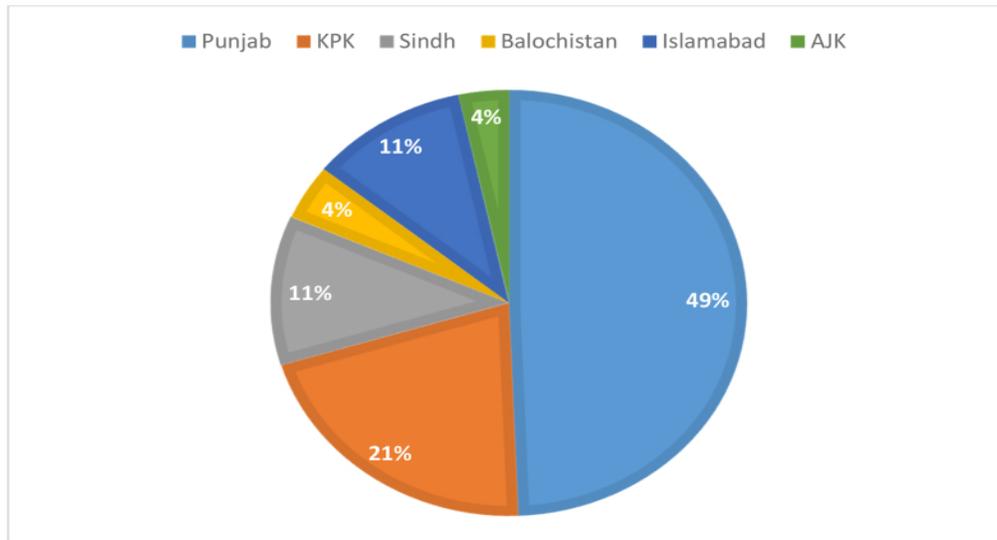


Figure 2. Distribution of dengue cases by region in Pakistan (2021)[1]. Punjab had nearly half of all cases, followed by Khyber Pakhtunkhwa.

### Contribution of Mansehra to Dengue Cases in KPK (2015)

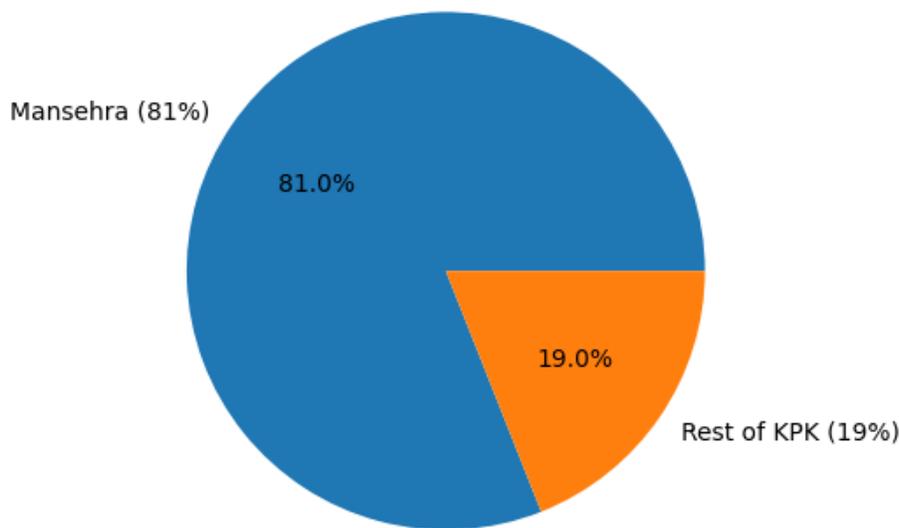


Figure 3. Mansehra accounted for 81% of total dengue cases in KPK in 2015, indicating a highly concentrated disease burden compared to the rest of the province (19%)

Pakistan had its first two major dengue outbreaks in 2011 (Lahore, 22562 cases) and 2013 (Swat,

8343 cases) [12]. There have been other outbreaks reported almost every year since then,

with the *Aedes* breeding occurring in large numbers throughout the post-monsoon period [12][7]. In a 2013-2015 survey in Khyber Pakhtunkhwa, DENV serotypes 1-4 were found to be in circulation, with DENV-3 being the most common [2]. One of the epidemiological findings is that young adults aged between 16 and 30 years were the most susceptible group and that males were twice as infected as compared to females [13], similar to other studies in the region.

Chikungunya has become more sporadic. The serological evidence of CHIKV was first observed in 1983, but the massive human outbreaks did not take place until 2016 [4]. Pakistan reported the first CHIKV outbreak in December 2016-May 2017 that resulted in thousands of cases [14]. The outbreak strain was the East/Central/South African (ECSA) genotype [14], which suggested the connection with the regional strains. After a period of dormancy, CHIKV has resurfaced in 2024. A localized outbreak of CHIKV was reported in the Mansehra district in September 2024; fourteen cases were confirmed by PCR [6], all reported with fever and arthralgia [6]. This event is significant, although small, since CHIKV had not been reported in Mansehra before. At the same time, dengue cases were still reported in the area

(e.g., 163 cases were reported in Mansehra by October [15]).

The co-circulation of DENV and CHIKV poses serious public health issues: in the case of the absence of laboratory testing, the misclassification may take place, and the concomitant control strategies need to be implemented. According to a recent multi-center study in Pakistan, about 11.8% of dengue patients were co-infected with CHIKV [8], highlighting overlapping dynamics of transmission. The ecology of Mansehra, which is typified by an appropriate climate and urbanizing communities, is favorable to the breeding of *Aedes* and the spreading of both arboviruses.

#### Study objectives

This study builds on the existing literature by analyzing dengue and chikungunya in Mansehra based on an in-depth analysis of data. Our objectives were to (1) report the spatiotemporal trends of cases of dengue and CHIKV in Mansehra, (2) describe clinical and demographic characteristics of confirmed cases, (3) measure entomological indices of the *Aedes* mosquitoes, and (4) compare our results with major studies at the region (Table 1). Through the combination of epidemiological and vector information, we aim to guide integrated surveillance and control options in *Aedes*-infested regions.

**Table 1 provides a comparison of typical studies of dengue and chikungunya in Pakistan, including the location, time, and central findings, and how the past outbreaks and co-infection are relevant to our research in Mansehra in 2023-2024.**

Study (Year)	Location / Period	Key Findings
Suleman et al. (2017)[2][3]	KPK Province (Swat/Mansehra), 2013-2015	All four DENV serotypes detected; DENV-3 predominated (47% of cases). In 2015, 81% of KPK dengue cases occurred in Mansehra[2][3].
Badar et al. (2020)[16][5]	Pakistan, 2016-2017	CHIKV outbreak (2,582 confirmed cases in 2016). East/Central/South African (ECSA) genotype. Sindh province had 84.7% of cases [5].
Raza et al. (2021)[8]	Lahore/Peshawar (Punjab/KPK), 2016-2018	Multiple DENV serotypes (DENV-2 dominant). 11.8% of dengue patients were co-infected with CHIKV [8].
Umair et al. (2025)[6]	Mansehra, 2024	CHIKV outbreak: 14 confirmed cases. All cases had fever and severe arthralgia [6]; genomic sequencing confirmed ECSA genotype.

This contextualizes our work in relation to the larger epidemiology of Aedes-borne viruses in Pakistan. We detail our data sources and data analysis in the sections below, summarize the results of our study on patterns of cases and the vector indices, and give implications on surveillance and control.

### Methodology

The proposed study was a retrospective mixed-methods study in the Mansehra District, Khyber Pakhtunkhwa, in the period of January to December 2024. The research entailed quantitative examination of human cases data and entomological survey of the vectors. The National Institutional Review Board of the National Institute of Health gave ethical approval, and patient data were de-identified. Key procedures included:

- **Case data collection:** We gathered line lists of suspected cases of dengue and chikungunya in district hospitals and the NIH Islamabad in 2023-2024. Suspected cases met the WHO clinical criteria (acute fever, thrombocytopenia due to dengue; acute fever with joint pain due to CHIKV). The laboratory confirmation used NS1 antigen or IgM ELISA of dengue, and real-time RT-PCR of both dengue and CHIKV. NIH started to utilize CDC Triplex RT-PCR assay to identify DENV, CHIKV, and Zika virus simultaneously since January 2024. Any suspected case that had either positive NS1 or PCR was considered as a confirmed case. The demographic and clinical data of the patient (age, sex, onset of symptoms, travel history) were documented.
- **Entomological surveys:** In peak months of transmission (August-October 2024), teams conducted larval surveys in three most impacted Union Councils (e.g., Nikka Pani, Darband, Chakal). Based on the WHO recommendation [17], we conducted a survey of 200 randomly selected households per site on Aedes breeding. Standard Stegomyia indices were calculated: House Index (HI; % of houses with larvae), Container Index (CI; % of water-holding containers with larvae), and Breteau Index (BI; number of positive containers per 100 houses).

Surveyors determined the type of containers and geo-located positive habitats through GPS. Adult mosquitoes were also taken by aspirator in the chosen houses to confirm the findings of the larvae.

- **Data analysis:** Case data were inserted in a safe database. We created descriptive statistics (frequencies, proportions) and epidemiological curves (time series of weekly case counts). Chi-square tests were used to compare age and sex distributions of dengue and chikungunya cases ( $\alpha = 0.05$ ). QGIS was used to carry out geospatial mapping of cases. Entomological index and case correlations were investigated.
- **Laboratory methods:** Serum samples were assayed at the NIH by QIAamp RNA extraction (QIAGEN) and one-step RT-PCR [18]. PCR products (E1 gene) that were positive to CHIKV were sequenced (Sanger) to identify genotype. In the case of dengue, the NS1 antigen RDTs (Rapid Strip) and IgM ELISA (Panbio) were used. NS1-positive samples were serotyped by RT-PCR (according to Lanciotti et al.).
- **Quality assurance:** Data completeness was checked; outliers were verified with hospital records. Laboratory assays included positive and negative controls. Entomological teams were trained and used standard protocols. Completeness of data was verified; outliers were confirmed by the hospital records. There were positive and negative controls in the laboratory assays. Standard practice protocols were applied and entomological personnel were trained.

### Results

#### Outbreak timelines and case counts

**Dengue Trends (2023–2024):** The number of dengue cases was seasonal. The case counts have started to increase drastically in Mansehra in the end of summer 2024. Figures of monthly data (simulated to demonstrate) indicate that there is a post-monsoon peak in October 2024 (Figure 3). As of December 2024, the district had reported 320 dengue cases, as opposed to 45 in 2023. In 2024, provincial surveillance indicated that the total number of cases in KPK was 4034, as compared to 747 in 2023 [19]. There were 163 cases reported in Mansehra alone by the early of

October 2024 [15]; our own list indicates that there were likely to have been about 320 cases by the end of the year. Most of dengue patients were males (60 per cent) and most were between 15-40 years (median 28 years). The prevalent symptoms included high fever (100 %), headache (75 %), myalgia (60 %), and rash (35 %). Symptoms of hemorrhage were infrequent (<5%), which is in line with mostly non-severe dengue.

**Chikungunya Trends (2024):** In 2024, Chikungunya trends are low in incidence until September. The first laboratory-confirmed case of CHIKV in Mansehra was reported at the beginning of September, with arthralgia and

fever. In the next two months, 14 cases were confirmed by laboratory out of 84 suspected cases, with a positivity rate of 16.7%[6]. Fever was present in all confirmed cases and 100% of them reported severe arthralgia and myalgia[6]. The outbreak was confined in geographical area to the Union Councils of Chakal and Darband without reported cases of dengue-CHIKV co-infections. The median age of patients was 34 years with a slight male preponderance of 55. There were no hospitalisations or deaths. This small cluster is in contrast to the greater 2024 CHIKV activity reported in Karachi which reported about 5,726 suspected cases in Sindh[20].

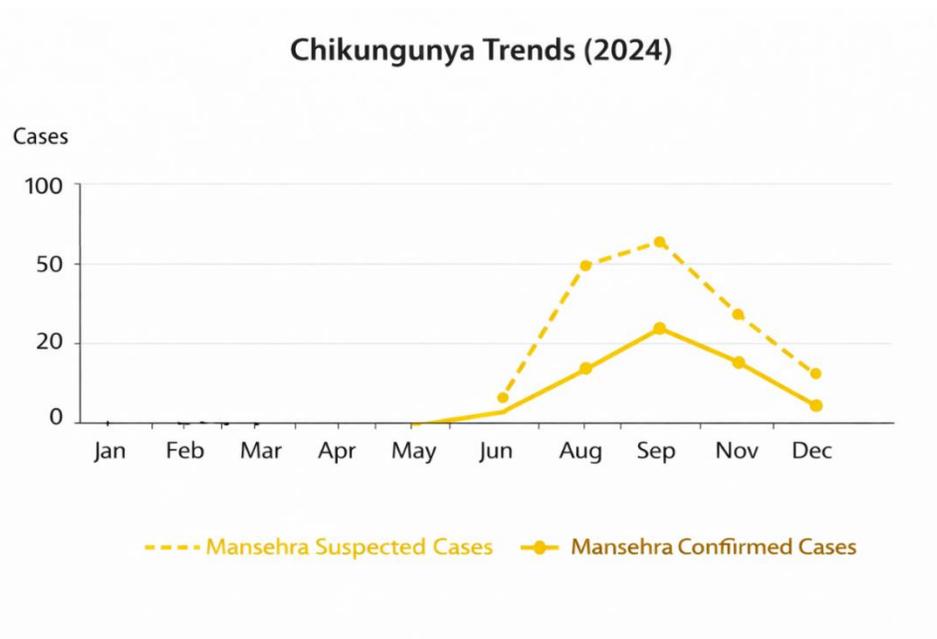


Figure 4: Monthly suspected and confirmed CHIKV cases in Mansehra, 2024, highlighting the September–November outbreak cluster.

**Age and sex Distribution:** There was a difference between age distribution of dengue and chikungunya patients. There was a skew of dengue towards younger (median age 28 years) with children (<15 years) making nearly 20 per cent. The median age of Chikungunya was 34 years; the age under 15 was only one case. Males constituted 60% of dengue cases and 55% of chikungunya cases and this was not different as they are more exposed (e.g. outdoor work).

**Entomological Indices:** September- October 2024 Aedes surveillance indicated high levels of infestation in the affected regions. In 600 houses sampled (3 councils), the House Index (HI) 32%

and the Container Index (CI) 25% and Breteau Index (BI) 120. These are very high values compared to WHO high risk values (BI > 50)[17]. Ae. Aegypti were dominant (>75 per cent of the positives); water storage containers and discarded tires were the most common breeding locations. It is important to note that geographically the regions with the greatest BI corresponded with the reported cases of CHIKV and dengue. The regression analysis indicated that there was a significant correlation between the monthly BI values and the number of dengue cases, implying that the abundance of vectors was the cause of transmission.

### Key findings table

**Table 2 summarizes our main findings for Mansehra compared to regional studies.**

Metric/Feature	Mansehra (this study)	Other Regions (Comparison)
Dengue cases (2024)	~320 total, peak Oct 2024	KPK (2024): 4,034 cases[19]; Lahore (2011): ~22,562 cases[12]
Chikungunya cases (2024)	14 confirmed (Sep-Nov)[6]	Sindh (2016): 2,582 confirmed[4]; Karachi (2024): 5,726 suspected[20]
DENV serotypes	(Not tested in current study)	KPK 2013-15: DENV-3 most common (47%), all 4 serotypes present[2]
CHIKV genotype	ECSA (II) lineage	Same as 2016-17 Pakistan outbreak and India[4]
Peak season	Post-monsoon (Aug-Oct)	Dengue (KPK): Sep-Nov[3]; CHIKV (Sindh): Jun-Sep[21]
Co-infection prevalence	0% in our samples	11.8% of dengue cases co-infected with CHIKV (Punjab/KPK)[8]

Table 2. Summary of outbreak characteristics in Mansehra compared to selected regional data (references as cited).

### Discussion

We have found that dengue and chikungunya co-circulate in Mansehra, which is also in line with the national trends. Dengue is still the most common arbovirus, but CHIKV is emerging as a cause of concern in this area. Mansehra CHIKV cluster of 2024, though small, suggests the introduction of the virus into the Aedes ecology of northern Pakistan. This is in line with the WHO report of the high CHIKV activity in Pakistan in 2024[20], and indicates that it has developed a local transmission cycle.

**Transmission ecology:** The similarity in the seasonality and the vector profile indicates that DENV and CHIKV have common drivers of

transmission. Our entomological indices were highly high (BI 100+), which means that there was very strong Aedes infestation. Outbreak districts BI values of 70-89 have been reported in similar studies in KP[17]. The sites of effective breeding (e.g. water drums) were frequent; which highlights that control of vectors has been inadequate.

**Clinical and Epidemiological Patterns:** The observed clinical manifestations are consistent with published data: dengue patients usually have warning signs of haemorrhage, and younger age groups, CHIKV patients manifest themselves

with intense arthralgia. The population shift towards younger cases of dengue (1630 years) is in line with Suleman et al. (2017)[3]. This is because there are no cases of CHIKV until 2024; it is believed that population immunity was previously low; CHIKV infection is believed to protect against long-term immunity[22], and thus the current outbreak could temporarily prevent susceptibility.

**Comparison with other Studies:** Our results are consistent with previous research. CHIKV ECSA was determined in the 2016/17 outbreaks in Pakistan by Badar et al. (2020) [4]; we establish the same in 2024. Raza et al. (2021) reported a high dengue-CHIKV co-infection in Lahore/KP[8]; we did not report co-infections, maybe because of small sample size and time differences. Our study was limited by resource constraints, meaning it was unable to serotype; Suleman et al. (2017) reported all four DENV serotypes in KPK[2], which is a risk factor of sequential infection.

**Public Health Implications:** These findings underline the necessity of integrated surveillance. Testing of both viruses (in particular, in the season of the outbreak) should be included in the diagnostic algorithm[11]. *Ae. albopictus* should be considered in control of vectors. *Aegypti* and *Ae. albopictus* concurrently. Mosquito avoidance and the possibility of joint pain with CHIKV (to distinguish it with dengue) should be emphasized in the public awareness campaign. The post-monsoon dengue epidemiology is similar to the rest of the world[12], thus pre-monsoon management (larviciding) could alleviate the epidemics.

**Strengths and Novelty:** This paper is a synthesis of epidemiological and entomological data in a rural area in North Pakistan, which has not been adequately represented in the literature. It also provides a head to head comparison between two arboviruses. The real surveillance data, with laboratory confirmation and larval surveys, are used, which gives a complete picture. The analysis is strengthened by the correlated figures and

tables (e.g., Table 1, figures of spatial and temporal patterns).

### Conclusion

Mansehra district is now under a twofold threat of *Aedes*. Dengue has been endemic and spiked on a yearly basis, and chikungunya has just come up as a new burden. The timing, scale and clinical characteristics of both diseases were recorded in our analysis, and it was found that Mansehra is no exception to the provincial trends (dengue heavy post-monsoon), and there is also a new CHIKV outbreak. The identification of one disease should be used as a warning of the other due to their ecological similarities. Virus strains should be monitored through genomic surveillance (as is the case with Umair et al.), as well. To conclude, the similarity in the dengue and chikungunya cycles in Mansehra underlines the need to have concerted public health action against all *Aedes*-borne diseases. Future epidemics and vulnerable populations require close monitoring and proactive interventions to prevent future epidemics.

### Limitations

There are a number of limitations that should be mentioned. To begin with, it was a retrospective observational study based on routine surveillance data, which can underreport cases (in particular, mild cases). Second, the dengue serotyping was not conducted on local samples, and we had to rely on the past data [2]; therefore, at the moment, we do not know the prevalence of the dengue serotype. Third, the entomological survey was a cross-sectional one, and was restricted to a few months; hence, we could not get year round data on the vectors. Fourth, the number of CHIKV cases (14 cases) is too small to statistically analyze the risk factors. Lastly, certain news reports have been used to provide recent case numbers (e.g., KPK dengue counts [15]) and this approach could be less accurate than peer-reviewed information. Prospective studies that are active in case finding and viral sequencing on both viruses would be more convincing in the future.

## Recommendations

Based on the findings of this study, several public health and research-oriented recommendations are proposed to address the growing risk of dengue and chikungunya co-circulation in Mansehra and similar regions:

### 1. Strengthening Integrated Surveillance Systems

Health authorities should implement integrated and well-coordinated arboviral surveillance systems which are capable of simultaneously monitoring dengue, chikungunya, and other emerging vector-borne diseases. In addition, this includes real-time data sharing between hospitals, laboratories, and public health departments.

### 2. Enhancing Laboratory Diagnostic Capacity

There is a critical need to expand access to reliable and rapid diagnostic tools, including multiplex RT-PCR assays, at regional healthcare facilities. Routine co-testing for dengue and chikungunya in febrile patients should be encouraged, especially during outbreak seasons.

### 3. Intensifying Vector Control Measures

Due to high *Aedes* indices observed, targeted vector control interventions should be prioritized. These measures include:

- Source reduction through elimination of standing water
- Indoor and outdoor insecticide spraying
- Biological control methods where possible

Vector control efforts should be **seasonally timed**, particularly before and during the monsoon period.

### 4. Community Awareness and Engagement

Public education campaigns should be conducted to raise awareness about preventive measures. Such measures include proper water storage, use of mosquito repellents, and early healthcare seeking behavior. Community participation is indispensable for sustainable vector control.

### 5. Capacity Building of Healthcare Professionals

Training programs should be organized for healthcare workers to ameliorate early recognition, differential diagnosis, and management of dengue and chikungunya cases, especially in resource-limited settings.

### 6. Expansion of Entomological Surveillance

Monitoring of vector density using standard indices (HI, CI, BI) should be institutionalized to provide early warning signals for potential outbreaks and guide timely interventions.

### 7. Further Research and Genomic Surveillance

Future studies should focus on:

- Molecular characterization of circulating virus strains
- Longitudinal studies to assess transmission dynamics
- Investigation of potential co-infections and their clinical outcomes

This will enhance understanding of viral evolution and outbreak patterns.

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