

DEMOGRAPHIC AND SEROPREVALENCE PATTERNS, OF DENGUE SEROTYPES IN DISTRICT DIR (L)

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Abstract

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Background: Dengue fever is a major arboviral threat, spread mainly by *Aedes aegypti*. It is a substantial public health challenge in Pakistan. In 2024, Tehsil Adenzai, District Dir Lower, had a notable dengue outbreak.

Objective: This study explores seroprevalence, demographics, and dengue serotypes during the 2024 outbreak in Tehsil Adenzai. The aim is to guide targeted control strategies.

Methods: We analyzed 87 confirmed NS1-positive cases. Blood samples were collected using WHO protocols. Samples underwent RNA extraction and RT-qPCR for serotype identification using standard lab methods.

Results: The outbreak showed clear patterns relevant to public health response. Males accounted for 56.3% of cases, and specific occupations had greater exposure. Most cases clustered in Ooch (19.5%), Bahram Shilman (18.4%), and Chakdara (14.9%), together contributing over half of the total. Adults aged 20–59 were predominantly affected (69%). DENV-2 was the leading serotype (40.2%), with DENV-1 (29.9%) and DENV-3 (9.2%) less common.

Conclusion: Dengue cases are grouped by location and demographic factors. This shows targeted interventions are important. DENV-2 is the most common and is linked to more severe illness, so better serotype monitoring is needed. Effective control will require focused vector management for high-risk jobs, community involvement, and ongoing serotype tracking.

Keywords: Dengue fever, Serotype distribution, Hotspots, Epidemiology, Pakistan, Vector control

INTRODUCTION

Mosquitoes, especially *Aedes aegypti*, are a major global health concern because they spread diseases like dengue, yellow fever, Zika, and chikungunya (WHO, 2020; Mallard et al., 2019). Dengue fever is rising quickly and now threatens more than 3.6 billion people. This is mainly due to rapid urban growth, poor waste management, and weak mosquito control in tropical and subtropical areas (Louis et al., 2014; Gubler, 1997). *Aedes aegypti* thrives in cities by breeding in small amounts of water found in discarded containers, tires, and storage pots (Rajkumar et al., 2010). The weather also plays a big role. Rain creates breeding sites, and warm temperatures help larvae develop faster, also increasing the risk of disease spread. These factors make control efforts more difficult (Byun & Webb, 2012; Tun-Lin et al., 2000). Pakistan is one country facing a growing dengue problem. Its subtropical climate, with seasonal rains and high temperatures, and fast, unplanned urbanization has led to a sharp increase in mosquito numbers (Haroon et al., 2018). Since the first reported case in 1960, Pakistan has seen a dramatic rise in dengue. There were over 75,000 confirmed cases in 2022 alone (Memon et al., 2024; Riaz et al., 2024). One of the biggest challenges is that mosquitoes have developed resistance to many insecticides, especially pyrethroids. This has made traditional chemical control less effective (Gupta et al., 2022). Because of this, there is a need to shift towards Integrated Vector Management (IVM). IVM uses targeted chemical control, basic environmental management like removing standing water from containers, and ongoing public education to encourage community involvement and better household practices (Ahamad et al., 2024; Campbell-Lendrum et al., 2015). Regular monitoring of mosquito populations and resistance patterns is also crucial. Continued monitoring will help keep these strategies effective and slow the spread of mosquito-borne diseases.

METHADODOLOGY

This study took place in dengue hotspots within Tehsil Adenzai, District Dir Lower, Khyber Pakhtunkhwa, Pakistan, an area known for frequent dengue outbreaks (Hayat et al., 2013). To identify and analyze the dengue virus, a thorough approach was used. Blood samples were collected from NS1-positive dengue patients using sterilized needles and EDTA tubes, adhering

to established safety protocols (WHO, 2012; CDC, 2010). After collection, samples were preserved and transported to the laboratory, where they were stored at 4°C for short-term and -80°C for long-term preservation to maintain viral RNA stability (Elcocks et al., 2014; WHO, 2011). For molecular analysis, RNA was extracted with TRIzol reagent, which involved chloroform extraction and centrifugation to separate phases (Thermo Fisher and Benchling, 2023). Following extraction, RNA was precipitated with isopropanol and washed with cold 75% ethanol to ensure purity (UConn Health, 2017). Next, serotyping and viral detection were performed using reverse transcription real-time PCR (RT-qPCR) with the SuperScript III Platinum One-Step qRT-PCR kit (Invitrogen). Primer concentrations were set at 300 nM for DENV-1, DENV-2, and DENV-3, and 450 nM for DENV-4 (Waggone et al., 2013). The amplification process included reverse transcription at 52°C for 15 minutes, followed by 45 cycles of denaturation and annealing, with fluorescence detection at 55°C to track amplification (Waggone et al., 2013). Positive results were identified using a set threshold within 40 cycles, while serotyping was conducted by detecting specific probe signals in the fluorescence channels for DENV-1 to DENV-4 (Waggone et al., 2013). Finally, the study followed strict ethical guidelines to protect patient confidentiality and properly handle sensitive data (WHO, 2011)

RESULTS

Tab: Epidemiological Profile of NS1-Positive Dengue Cases (2024) - Tehsil Adenzai, Dir Lower

Area	Total Cases	Adult (20-59)	Adolescent (13-19)	Child (0-12)	Elderly (60+)	Male	Female	Dominant Serotype(s)	Primary At-Risk Occupation
Ooch	17	14	2	1	3	11	6	DENV-1	Outdoor Laborers, Farmers
Bahram Shilman	16	7	5	2	2	10	6	Unidentified	Outdoor Laborers, Farmers

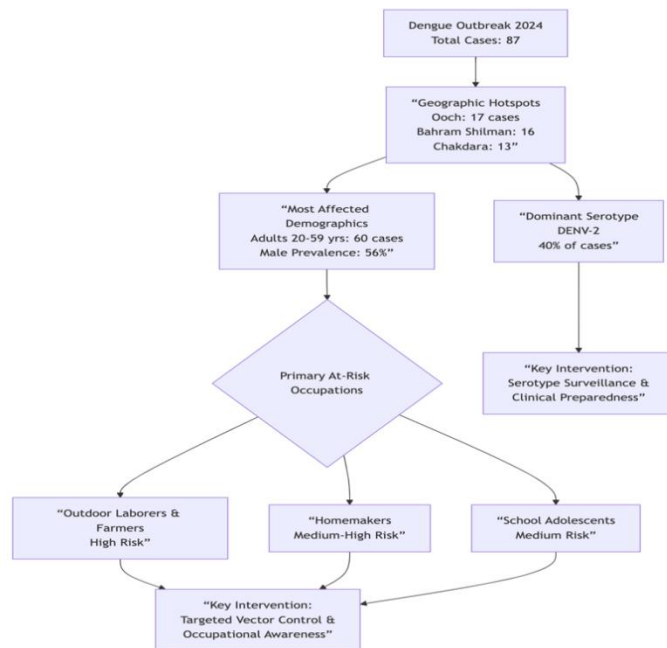
Chakdara	13	6	2	5	0	7	6	DENV-2	Homemakers, Outdoor Laborers
Shawa	11	5	4	1	1	6	5	DENV-2	School Adolescents, Homemakers
Gull Abad	8	4	3	1	0	4	4	DENV-2	School Adolescents
Ramora	6	5	1	0	0	4	2	DENV-1	Outdoor Laborers
Badwan	5	4	1	0	0	4	1	DENV-3	Outdoor Laborers
Tendodag	5	4	1	0	0	3	2	DENV-2	Outdoor Laborers
Osaki	3	3	0	0	0	2	1	DENV-1	Outdoor Laborers
Khadagzoo	3	2	1	0	0	2	1	DENV-3	Outdoor Laborers
TOTAL	87	60	19	10	9	49	38	DENV-2 (40.2%)	All Groups
% of Total	100%	69%	21.8%	11.5%	10.3%	56.3%	43.7%	-	-

Geographic Hotspots: Ooch, Bahram Shilman, and Chakdara are the primary outbreak epicenters, accounting for over 52% of all cases.

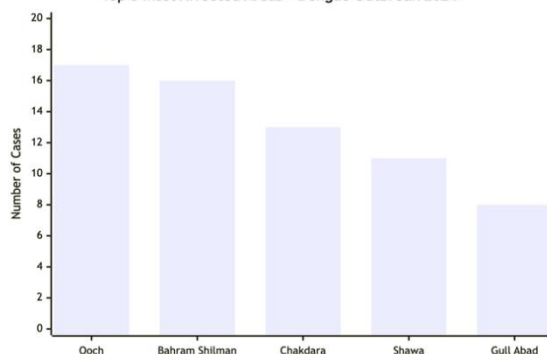
Demographic Impact: The outbreak predominantly affects working-age **Adults (69%)**, with a significant secondary impact on **Adolescents (21.8%)**. There is a clear male predominance (56.3%).

Serotype Dynamics: **DENV-2** is the dominant and most widespread serotype. DENV-1 is concentrated in specific areas (Ooch, Ramora, Osaki), while DENV-3 is highly localized. A significant cluster of unidentified serotypes was found in Bahram Shilman.

Occupational Risk: The data clearly shows that **Outdoor Laborers and Farmers** (primarily adult males) are at the highest risk. **School-going Adolescents** and **Homemakers** form other significant risk groups, indicating transmission in both outdoor and peri-domestic environments



Top 5 Most Affected Areas - Dengue Outbreak 2024



DISCUSSION

The dengue virus, spread by *Aedes* mosquitoes, continues to pose a serious public health risk worldwide. Its transmission depends on many factors: climate, population density, and human behavior (Gubler, 2011). Pakistan, with its varied climate, has experienced several outbreaks. These have been particularly notable in Khyber Pakhtunkhwa (KP) (Khan et al., 2020). In 2024, Tehsil Adenzai in Lower Dir district, KP, reported a notable dengue outbreak. There were 87

confirmed NS1-positive cases. While this number is much lower than the 24,807 cases from the previous year, it still highlights ongoing health challenges (Hayat et al., 2013). More men were affected, making up 56.3% of cases. This is likely because many men in the area work outdoors in jobs like farming and construction. These roles increase their exposure to mosquitoes (Harapan et al., 2020). Most cases were concentrated in three areas: Ooch (19.5%), Bahram Shilman (18.4%), and Chakdara (14.9%). Together, these accounted for over half of all cases. These locations have conditions that help mosquitoes breed, such as standing water and poor waste management. This shows the need for focused mosquito control efforts like spraying and larviciding (WHO, 2020). The age breakdown showed that adults aged 20 to 59 were most affected. They made up 69% of cases, likely due to their outdoor work (Lahariya & Pradhan, 2006). Teenagers (13–19 years) made up 14.9% of cases. Children and older adults had fewer cases, probably because they spend less time outside (Pender, 2011). Among the virus types, DENV-2 was the most common (40.2%), followed by DENV-1 (29.9%) and DENV-3 (9.2%). DENV-2 is especially worrisome because it is linked to more severe illness, such as dengue hemorrhagic fever and shock syndrome (Gubler, 1997). Mixed serotypes were found in 20.7% of cases. This makes treatment more complicated and shows why ongoing monitoring of virus types is important for public health planning (Louis et al., 2014).

Conclusion,

Dengue outbreak in Tehsil Adenzai illustrates the intersection of occupational, geographic, and demographic risk factors. Effective control requires targeted measures, including enhanced vector control in identified hotspots, public awareness campaigns for high-risk groups such as outdoor workers, and robust serotype-specific surveillance to mitigate future outbreaks

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this study.

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Authors' Contributions

Bahar Uddin: Conceptualization, data curation, and manuscript writing. **Jalal Ud Din, Masood, and Wasi Ullah:** Investigation, methodology, **Amjid Akhtar, Muhammad Sulaman Shah, and Asiya Khan:** data analysis. **Fawad Khan:** Supervision, project administration, validation, and final manuscript review.

All authors reviewed and approved the final version of the manuscript.

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