

# Epidemiological trends of mosquito-borne viral diseases in Pakistan



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## Abstract

Globally, arboviruses are public health problems. Pakistan has seen a fast-paced increase in mosquito-borne *Flavivirus* diseases such as dengue because of deforestation, climate change, urbanization, poor sanitation and natural disasters. The magnitude and distribution of these diseases are poorly understood due to the lack of a competitive nationwide surveillance system. In dengue-endemic countries, the recent epidemics of chikungunya (CHIKV) and human West Nile virus (WNV) have created panic among the public and are thought to provoke an outbreak of Zika virus (ZIKV) in Pakistan. Recently, hospital-based surveillance has indicated the presence of Japanese encephalitis virus (JEV), which is deeply concerned by developing countries such as Pakistan. The situation could become more devastating because of poorly developed diagnostic infrastructure. To date, no licensed vaccine has been used in Pakistan, and preventive measures are mainly based on vector control. This review provides comprehensive information concerning the association of risk factors with disease occurrence, epidemiological trends, and prediction of the spread of mosquito-borne diseases, attention to new threats of ZIKV, and future perspectives by benchmarking global health policies.

**Keywords:** Arboviruses, Dengue, CHIKV, JEV, WNV, ZIKA, Public health

## Introduction

Mosquito-borne diseases (MBDs) are common in tropical and subtropical countries and cause substantial public health issues (World Health Organization 2016). According to the world health organization (WHO), one-sixth of the global population is affected by vector-transmitted diseases, and among them, dengue fever is the world's fastest-growing disease, with a 30-fold increase in incidence over the last 50 years. Several factors, including deforestation, climate change, human mobility, migratory bird patterns, disordered occupation of urban

territory, and changes in vector ecology, may influence the expansion of MBDs to nonendemic areas (Campbell-Lendrum et al. 2015).

Pakistan is located in the convergence of South Asia, Central Asia and West Asia which is important geographically. It is well characterized by its geographical and climate diversity and links the East with the West. Pakistan has the largest cargo port of South Asia, which is located on the Arabian Sea. Most imports and exports go through this port. Therefore, there is a possibility of spreading infections during trading between neighboring countries.

Pakistan has faced serious problems of vector-borne diseases such as Crimean-Congo hemorrhagic fever, leishmaniasis, dengue hemorrhagic fever, chikungunya and West Nile fever because of its climatic suitability for vectors and subtropical location (Bostan et al. 2017). Dengue and other vector-borne diseases have been

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reported during the last ten to fifteen years, but chikungunya, Japanese encephalitis and West Nile fever arrived fairly recently in Pakistan and are restricted to some parts of the country. Now the question is whether chikungunya virus (CHIKV), West Nile virus (WNV) and Japanese encephalitis virus (JEV) will spread to the rest of the country like Dengue fever, and whether Zika virus will be a future threat for Pakistan.

This review provides answers to these questions in addition to risk factors, the epidemiological status of important MBDs in Pakistan, forecasts about disease distribution in new areas of the country, upcoming Zika virus (ZIKV) threats, and future perspectives by benchmarking world health policies.

## Factors leading to disease spread in Pakistan

### Climate

There is a strong association between climate change and mosquito-borne diseases. As the ideal temperature (26–29 °C) is reached, vector activities increase, which leads to more disease spread (Mordecai et al. 2017). Above 16 °C, most tropical mosquitoes complete their life cycle. According to data from “Trading Economics”, Pakistan’s average temperature from December to March is 10 °C (<https://tradingeconomics.com/pakistan/temperature>). A study has been published to determine the relationship between temperature and the total number of mosquitoes collected monthly in Lahore, Pakistan, and they found that from November to March, when the temperature was below 16 °C, mosquito collection was almost zero. These data indicate that temperature is a critical factor for mosquito proliferation, which ultimately impacts the prevalence of MBDs in Pakistan (Manzoor et al. 2020).

Like other Asian countries, Pakistan also faces substantial climate variation. Every year, the temperature rises, the summer becomes warmer, and winter is not as cold as before (Fahad and Wang, 2020). This increasing temperature has nurtured the epidemic of many MBDs. Pakistan also faces heavy monsoon rainfall every year that leaves water pools and puddles, which ultimately become the breeding grounds for mosquitoes (Rasheed et al. 2013).

During 2010–2012, Pakistan suffered from its worst natural disaster of 3 consecutive floods, which resulted in the loss of human lives, properties, and agricultural land. Twenty million people were affected by these floods, and moved to camps for temporary settlement. Overcrowding, inadequate drinking water, poor sanitation, and increased exposure to mosquitoes caused dengue and other infections (Saeed and Piracha 2016). In 2010–2011, the average temperature of Pakistan was higher than that in the last 3 decades, and the largest number of dengue cases was recorded all over the country (Fig. 1a). WNV cases and chikungunya outbreaks

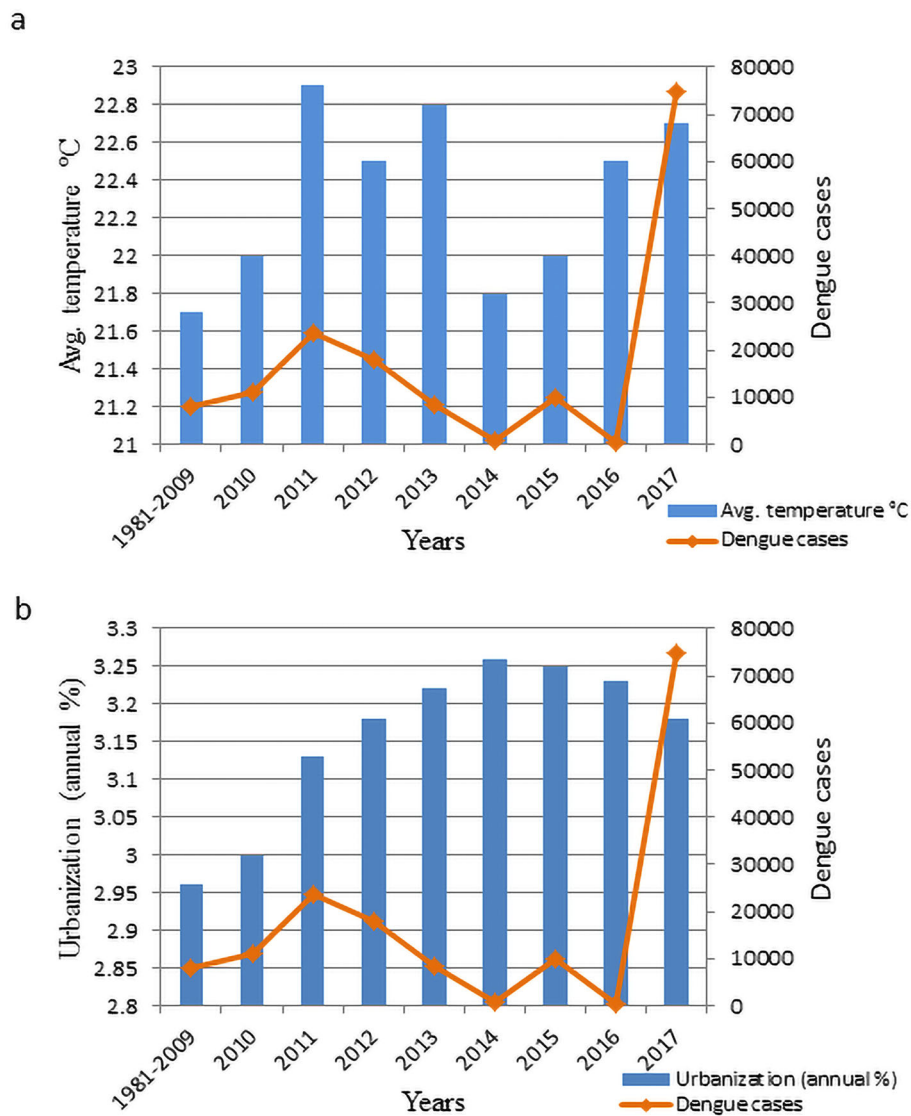
were reported in Karachi in 2015 and 2016, respectively. Important factors included the warm and humid environment of the city along with poor sanitary conditions. The temperature of Karachi city in winter is over 15 °C, so mosquitoes quickly proliferate in this conducive environment (Rauf et al. 2017). In 2010–2011, the surveillance data of temperature and precipitation revealed a slight increase in temperature in Pakistan during January and February. This means that the climate of Pakistan is most suitable for vector-borne diseases.

### Urbanization and socioeconomic factors

Urbanization and colonization are the results of economic development. Many problems, such as environmental pollution, destruction of natural ecology, and overcrowding, have been associated with urbanization. There are direct and indirect effects of urbanization on vector biology and vector-borne disease transmission (Li et al. 2014). *Aedes aegypti* (*A. aegypti*) and *Aedes albopictus* (*A. albopictus*) are anthropophilic mosquitoes. Massive movement to urban areas for industrialization in Pakistan led to the expansion of these mosquitoes, which resulted in an increased risk of disease outbreaks. The emergence of dengue virus (DENV) coincides with urbanization. Interestingly, the DENV-2 strain is more prevalent in Pakistan and other Asian countries (Bostan et al. 2017). With urban development, artificial water sources, including underground water tanks and water holding pots, provide breeding grounds for vectors (Wu et al. 2009). Substandard socioeconomic status is also an important risk factor. In 2009–2012, the annual % of urbanization increased in Pakistan, and the maximum number of cases was reported in these years from all parts of the country (Fig. 1b).

### Globalization, travel and trade factors

Vector-borne diseases can reach many areas of the world by travel and the transport of goods and can become an epidemic first and then endemic. *A. aegypti* first originated in Africa and then spread globally. It was hypothesized that *A. aegypti* was introduced into the New World due to the harsh environment of Africa coupled with the slave trade and then spread to the Indian and Pacific Ocean. Currently, both *A. aegypti* and *A. albopictus* are present in Asian countries (Kraemer et al. 2015). It was also hypothesized that the transport of used tires in containers between many countries during 1978–1985 contributed to the worldwide dispersal of *Aedes* mosquitoes (Reiter and Sprenger 1987). In 2000, a survey was conducted by Peshawar University and found *A. aegypti* in tires stored in warehouses. After this, all Karachi regions were resurveyed for the presence of mosquitoes, and the same results were found with a greater distribution of *A. aegypti*, indicating *Aedes* dispersal in Pakistan



**Fig. 1** A graphical representation of the association of temperature (a) and urbanization (b) with dengue cases. Temperature data were collected from 'Annual report of Pakistan Meteorological Department' (<http://www.pmd.gov.pk/>) and urbanization annual % data from 'Pakistan Urban Population Growth Annual Percent-Trading Economics' (<https://tradingeconomics.com/pakistan/urbanpopulation-growth-annual-percent-wb-data.html>)

associated with the tire trade (Suleman et al. 1996). The government should take measures to control and check international movement by implementing strict health criteria for viral and other diseases for people entering the country.

**Unhygienic conditions**

Deplorably unhygienic conditions further acted as fuel for the fire by providing a suitable environment for mosquito proliferation. Sanitation in Pakistan is characterized by some achievements and many challenges. Despite high population growth, the share of the population with access to an improved water source has grown

from 37% in 1990 to 78% in 2020, although this does not necessarily mean that the water from these sources is safe to drink. The share of the population with access to improved sanitation increased from 27% to 48% during the same period, according to the Joint Monitoring Program for Water Supply and Sanitation ([https://en.wikipedia.org/wiki/Water\\_supply\\_and\\_sanitation\\_in\\_Pakistan#cite\\_note-ADB\\_11-10](https://en.wikipedia.org/wiki/Water_supply_and_sanitation_in_Pakistan#cite_note-ADB_11-10)). Nevertheless, Pakistan is among the top 10 countries in the world that lack access to clean water. Seventy-nine million people in Pakistan do not have access to a proper toilet. Water-borne diseases are prevalent as a result of untreated drinking water. These unhygienic conditions in Pakistan

further aggravate the dispersal of vectors that are responsible for disease outbreaks (<https://borgenproject.org/sanitation-in-pakistan/>). Unhygienic conditions and a lack of municipal services cause trash and water accumulation in residential areas, which aids larval growth. In addition, improper vector control surveillance, the use of disposable items, and improper disposal of tires enhance hyperendemicity of vectors and vector-borne diseases (Roberts and Kemp 2001).

### Geographical distribution of mosquitoes

The geographical distribution of mosquitoes in different locations is also a critical factor for disease epidemics. The species and the number of mosquitoes that carry viruses were surveyed at the district level in different provinces in Pakistan. Both *A. aegypti* and *A. albopictus* were present in almost all the dengue-affected provinces of Pakistan. Although *A. albopictus* was detected in more locations, both species were detected in the urban areas of central Punjab most impacted by dengue (Ashfaq et al. 2014). In Khyber Pakhtunkhwa, a total of 4086 adult mosquitoes were identified, comprising 11 species belonging to four genera: *Culex*, *Aedes*, *Anopheles*, and *Armigeres*. Among all the species, *Cx. quinquefasciatus*, *Cx. tritaeniorhynchus*, *Cx. vishnui*, and *A. albopictus* were the dominant species (Wajiha et al. 2017). The mosquito fauna of Balochistan Province is unknown. Numerous cases of DENV have been reported in this province. There is no available information on the geographical distribution of mosquitoes at the local level in Pakistan.

### Epidemiological trends of mosquito-borne disease Dengue fever

The WHO reported that approximately 124 countries are affected by DENV, and in these countries, approximately 2.5 billion people (almost one-third population of the world) are considered at high risk. Every year, more than 50 million people are susceptible to this arbovirus infection, and a 2.5% mortality rate has been recorded (World Health Organization 2011). The genus *Aedes* of mosquitoes is responsible for the transmission of the dengue virus DENV, and the most prominent vector is *A. aegypti* due to its anthropophilic nature (Wang et al. 2000).

In Pakistan, the first documented outbreak of dengue was reported in 1994 in Karachi, in which 1000 people were infected. This outbreak occurred from August to November, and 2 people died (Chan et al. 1995). To investigate this outbreak, many studies have been conducted using an enzyme-linked immunosorbent assay (ELISA) to detect immunoglobulin M (IgM) against DENV-1 and DENV-2 and a seminested polymerase chain reaction PCR. In 1995, dengue was also confirmed

in patients suffering from pyrexia due to mosquito bites in west Karachi by detecting IgM against serotypes 1 and 2 (Ahmad et al. 2017).

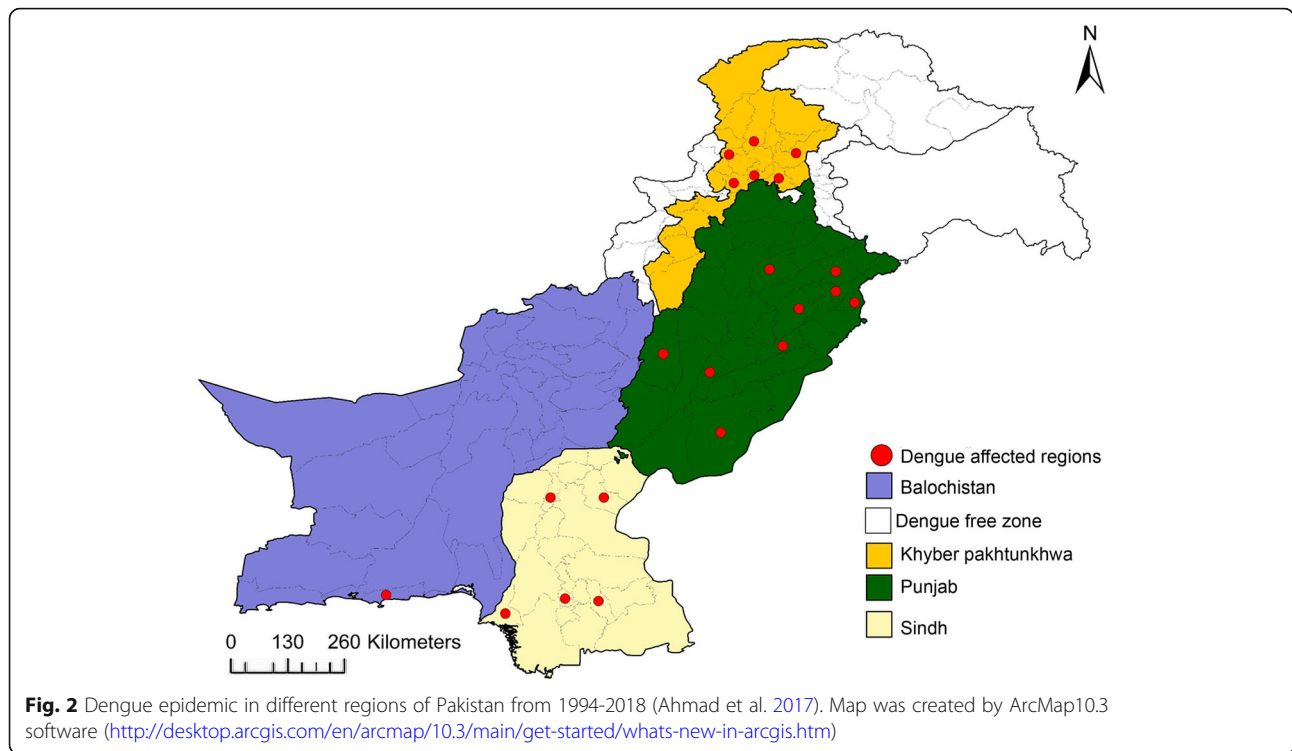
In 2005, after 10 years without cases, 395 cases of dengue from Karachi were confirmed positive by the National Institute of Health, Islamabad. In the same year, many patients were hospitalized in Karachi with clinical signs that were consistent with the WHO definition of DENV. Out of 106 patients who were clinically positive for DENV, nine died. Forty-two samples from 106 were dengue IgM positive. Six out of nine patients who died had both IgM and IgG dengue antibodies, while three patients had only IgM. Furthermore, through RT-PCR, these three patients who died were confirmed to be dengue positive. A sequence analysis of the PCR product showed it was serotype 3. This serotype was significantly similar to Indian dengue serotype 3, which was isolated from Delhi (Jamil et al. 2007).

Before 2006, dengue was not commonly seen across Pakistan. It was only restricted to the Karachi area. However, from 2006-2017, due to the movement of people infected with dengue to areas without dengue infections and the presence of vectors in these regions, there was an outbreak of dengue in all provinces in different years (Fig. 2 and Table 1), and it is now endemic in the whole country (Li et al. 2014). In Khyber Pakhtunkhwa, dengue became uncontrollable in 2017 and affected 74820 people. Fifty-four people died out of 15828 confirmed cases. In this outbreak, the prevalent serotypes were serotype 1 (6%), serotype 2 (33%), serotype 3 (47%) and serotype 4 (0.1%) (Suleman et al. 2017).

### Chikungunya

The word chikungunya is derived from the Makonde language and means “to become contorted”, which refers to a stooped appearance. This posture is due to arthralgia, which is the characteristic sign of this illness. Other symptoms include high fever, nausea, headache, rash, myalgia, and vomiting. It also affects the nervous, pulmonary, cardiovascular, cutaneous and ocular systems as sequelae of infection (Rajapakse et al. 2010; Robinson 1955; Ali and Dasti 2018).

In Pakistan, chikungunya was first detected in 1983 in the sera of rodents and humans. However, at that time, no spillover of the virus occurred (Darwish et al. 1983). A few cases of chikungunya were also identified during the outbreak of dengue in 2011 (Halstead 2015). Despite the clear evidence of CHIKV, little attention was given regarding its monitoring and control strategies, which caused its reemergence in Karachi in November 2016. Other factors, including climate change and poor sanitation and health care infrastructure, were linked to this outbreak. According



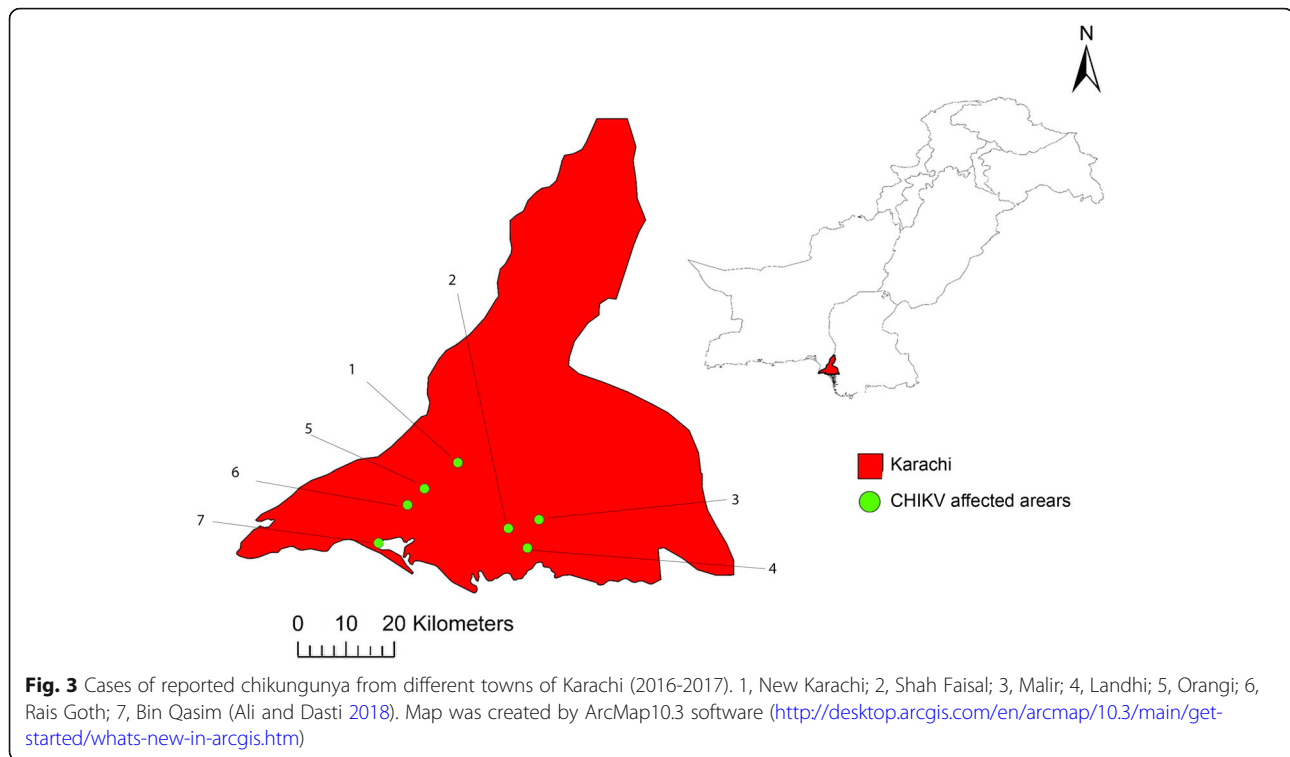
to the reports of different health care authorities, more than 30000 people were affected. The National Institute of Health (NIH), Islamabad and the Armed Forces Institute of Pathology confirmed more than 4000 CHIKV-infected people by using qualitative RT-

PCR. This outbreak continued until April 2017 (Afzal et al. 2015). The affected towns were Shah Faisal, Malir, Orangi, Bin Qasim, Rais Goth, Landhi, and New Karachi (Fig. 3). Later, the number of cases of CHIKV was denied, and according to a subsequent

**Table 1** Dengue-infected cases, deaths, and prevalent serotypes from affected areas of Pakistan (Ahmad et al. 2017)

Year	Cases	Deaths	Affected provinces	Prevalent serotype
2008	2469	17	Sindh, Punjab, Khyber Pakhtunkhwa	DENV-2, DENV-3, DENV-4
2009	1085	13	Sindh, Punjab, Khyber Pakhtunkhwa	DENV-2, DENV-3
2010	11024	40	Sindh, Punjab	DENV-1, DENV-2
2011	23552	219	Sindh, Punjab, Khyber Pakhtunkhwa	DENV-2, DENV-3, DENV-4
2012	18000	350	Sindh, Punjab, Khyber Pakhtunkhwa	N/A
2013	8546	33	Khyber Pakhtunkhwa Baluchistan	DENV-1, DENV-2, DENV-3, DENV-4
2014	584	N/A-	Sindh, Punjab	DENV-1, DENV-2, DENV-3
2015	9899	07	Sindh, Punjab, Khyber Pakhtunkhwa	DENV-1, DENV-2, DENV-3, DENV-4
2016	340	-	Sindh	DENV-1, DENV-2, DENV-3, DENV-4
2017	74820	54	Khyber Pakhtunkhwa, Sindh, Punjab, FATA, Baluchistan	DENV-1, DENV-2, DENV-3, DENV-4

Notes: DENV, dengue virus. Data were collected from the National Institute of Health Islamabad and WHO country office in Pakistan (<https://reliefweb.int/disaster/ep-2013-000136-pak>, <http://outbreaknewstoday.com/pakistan-reports-10000-dengue-cases-in-2015/>, <https://reliefweb.int/map/pakistan/pakistan-dengue-outbreak-sindh-1-January-march-31-2016>, [http://applications.emro.who.int/docs/epi/2017/Epi\\_Monitor\\_2017\\_10\\_36.pdf](http://applications.emro.who.int/docs/epi/2017/Epi_Monitor_2017_10_36.pdf))



report of the NIH of Pakistan, 818 cases were suspected, and 82 cases were laboratory-confirmed (Aamir et al. 2017).

Pakistan had already experienced viral outbreaks, including Crimean-Congo hemorrhagic fever and dengue. Currently, CHIKV infection has attracted the attention of government health departments and the WHO. Epidemiologists linked the outbreak of CHIKV in Pakistan with an epidemic in India a few months before. The NIH issued an alert after the outbreak of CHIKV in India, but no preventive measures were taken at railway stations, airports and the Pakistan-India border; ultimately, Pakistan was affected in September 2016 (Aamir et al. 2017; Mallhi et al. 2017).

#### West Nile fever

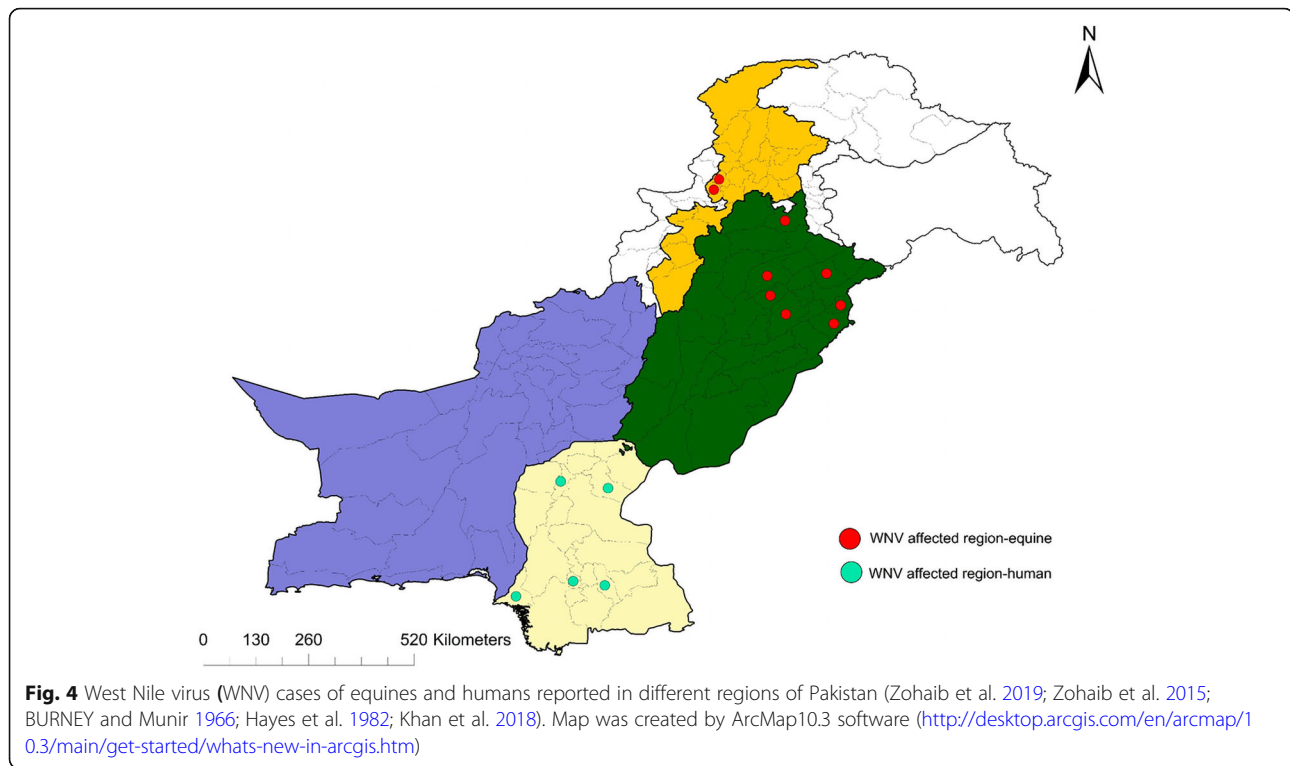
WNV is an important member of the genus *Flavivirus*. It has a mosquito-bird transmission cycle and has been detected in 65 species of mosquitoes and 326 species of birds in the US. *Culex* mosquitoes are responsible for its transmission (Komar et al. 2003). Humans are unlikely to be infected by WNV and are considered dead-end hosts due to the low level of viremia in human serum (Aamir et al. 2017). WNV induces neuroinvasive disorder (encephalitis, meningitis, acute flaccid paralysis) along with fever (Zou et al. 2010).

In 1966, WNV was isolated from human blood and mosquitoes in the Rawalpindi area of Pakistan, and it was antigenically similar to the Egypt 101 strain

(BURNEY and Munir 1966; Hayes et al. 1982). In 1978-1979, a seroepidemiological study was conducted in Punjab Province with samples from domestic and wild animals from Changa Manga forest and Chiniot region against WNV (Fig. 4). Eighty-five samples out of 317 wild birds were WNV antibody positive. The mammal most commonly used for these studies was the Indian cow, and out of 58 samples, 21 were positive for WNV antibody (Hayes et al. 1982). During 2012-2013, 449 equine samples from two provinces (Punjab and Khyber Pakhtunkhwa) of Pakistan were tested against WNV and other flaviviruses by using ELISA (Fig. 4). The positive samples were further investigated by using a virus-specific microneutralization test. A total of 249 samples out of 292 ELISA-positive samples were confirmed to have only WNV infection, which suggests the high prevalence of WNV in equines (Zohaib et al. 2015). This presented the possibility of WNV in humans.

In 2015-16, Khan et al. performed a serological study of 997 suspected arboviral cases in humans who suffered from altered mental status, reduced Glasgow Coma Scale scores, and seizures (Fig. 4). They found that 105 patients were IgM positive and 71 had neutralizing antibodies against WNV. This was the first study of WNV-associated neurological disease in humans in the Sindh Province of Pakistan (Khan et al. 2018).

In 2019, active circulation of WNV lineage 1 was identified in asymptomatic patients of blood donors in



Punjab Province. This means that it can be transmitted during blood transfusion, and it indicates the urgent need for surveillance of WNV to determine its distribution and impact in Pakistan (Zohaib et al. 2019).

#### Japanese encephalitis (JE)

Most human JEV infections are asymptomatic but can range from mild fever to life-threatening encephalitis (Imran et al. 2019). JE is a vaccine-preventable disease, and it causes meningoencephalitis and flaccid paralysis in one out of 300 cases. According to the WHO, only 10% incidence was reported annually in JE-endemic areas (Campbell et al. 2011).

In the 1980s and 1990s, studies were conducted in Karachi based on nucleic acid detection in encephalitis patients, which revealed for the first time the incidence of JE in Pakistan (Sugamata et al. 1988). In 2018, Khan et al. detected immunoglobulin M (IgM) of JEV in patients suffering from febrile illness in southern Punjab, Pakistan. In 2020, a study was published based on JE surveillance in hospitalized patients admitted from 2015-2018 to two acute care hospitals in Karachi to investigate whether JEV is a cause of acute encephalitis in Pakistan. Cerebrospinal fluid and serum samples were tested for JEV-IgM. Six out of eight patient samples were positive for JEV-IgM. Meanwhile, patients also showed serological cross-reactivity to WNV and DENV (Fatima et al. 2020). Thus, this study could not confirm or exclude local JEV transmission. Furthermore, this was not a

population-based study and thus could not provide exact data on JE incidence. This study also captured a lower proportion of children, who are considered the most susceptible to JEV infections (Solomon 2004). In Pakistan, JEV diagnosis is complicated due to the presence of cocirculating flaviviruses, the presence of vectors, and the amplification of hosts in endemic areas. There is a need for a nationwide surveillance system and the establishment of fully equipped diagnostic labs to confirm JE infections

#### Will MBDs spread to new regions of Pakistan?

In developing countries such as Pakistan, it is difficult to control vectors where infrastructure is poorly developed. Additionally, every year, the temperature is increasing in Pakistan. With increasing population density, movement of people, and the transport of animals, goods and agricultural products, the spread of vectors into new regions of the country seems probable, particularly causing the spread of CHIKV, WNV and JEV. For the last ten years, DENV has affected almost every province of the country. Currently, CHIKV, WNV and JEV have been reported in Sindh Province, mainly in Karachi. Karachi is one of the largest industrial cities of Pakistan, and people move from all over the country to this city for employment and education. During national holidays, they travel back to their home cities.

If we look at the history of dengue outbreaks, it seems that *Ae. albopictus* and *Ae. aegypti* were not reinvading

the same region (e.g., Karachi, Lahore and Peshawar) but spreading to new areas (e.g., Rawalpindi, Taxila, Gujranwala, Attock, Haripur, Faisalabad, Multan, Sheikhpura, Hasanabdal and Hyderabad) by the transportation of containers and old tires (Khan, Khan and Amin 2016, Wu et al. 2009). This will be the cause of the spread of CHIKV, WNV and JEV to other parts of the country. In addition, the Khyber Pakhtunkhwa province has mountainous cities, and people visit these areas from different parts of Sindh and Punjab, which can be a source of disease spread to new geographic regions. Migratory birds can also play an important role in the spread of WNV to different parts of the country. This statement is supported by data on WNV movement in the Old World by migratory birds (Malkinson et al. 2002).

Additionally, WNV was also reported in equines in some parts of Punjab and Khyber Pakhtunkhwa, which presents the possibility of WNV transmission in humans in these provinces. Furthermore, Lahore borders India, and the serotype isolated from the dengue epidemic in Lahore was similar to the serotype prevailing in India (Fatima et al. 2020).

Thus, we cannot rule out the possibility of the introduction of other mosquito-borne viruses from countries bordering to unaffected regions of Pakistan. Furthermore, Karachi houses the largest cargo port of South Asia, located on the coastline of the Arabian Sea. Therefore, there might be a spread of infections during trading among neighboring countries. Hence, national and international health organizations must address this serious issue to quell this plague in time (Reiter and Sprenger 1987).

### Will ZIKV be a future threat for Pakistan?

ZIKV is an emerging mosquito-borne virus similar to CHIKV and DENV in terms of clinical signs and transmission vectors. Both *Ae. aegypti* and *Ae. albopictus* are now present in large cities of Pakistan. Despite control strategies, breeding sites are still found in the country.

In addition to sharing similar clinical signs with DENV and CHIKV, ZIKV is associated with microcephaly and Guillain–Barré syndrome (GBS) (Hughes and Cornblath 2005). Recently, many cases of neonatal microcephaly and GBS of unknown etiology were reported in Pakistan (Qasim 2016), although there were no reported confirmed ZIKV cases. In 1983, a serological study indicated the presence of ZIKV antibodies in the sera of rodents, humans and domestic animals (Darwish et al. 1983). One of the challenges in ZIKV detection is that more than 60% population lives in rural areas, and people do not consider visiting hospitals if they suffer from mild symptoms such as fever, conjunctivitis, joint pain, and rash. Medical officers are also more inclined

to identify dengue cases due to endemicity from the last 10 to 12 years. There is no diagnostic kit for ZIKV at the local practitioner's level, so proper screening is a challenging task (Butt et al. 2016). According to the Global Outbreak Alert and Response network, the vehicle of ZIKV is present in Pakistan (Wahid et al. 2016). ZIKV laboratory confirmation can also show evidence of cross reactivity with DENV and other *flaviviruses*.

Apart from this, cases were also reported in countries sharing a border with Pakistan: India and China. In May 2016, the health ministry of India reported three confirmed ZIKV cases in the western state of Gujarat, which is close to the Sindh Province border of Pakistan. In February 2017, China also reported an imported ZIKV case of a person in Henan Province who had been working in Central America for two months (Dhimal et al. 2018). These findings suggest the presence of ZIKV in the southeast region of Asia, and if we look at the history of dengue epidemics, then there are chances of ZIKV emergence in Pakistan (Wahid et al. 2016).

Previous outbreaks of *flaviviruses*, climatic conditions, mosquito biology, and travel patterns predict that the next landing spot of ZIKV is Pakistan. An explosive epidemic is expected to occur in two large cities (Karachi and Lahore) of Pakistan because of rapid growth in slum areas and vector populations (Khan et al. 2016). In September 2016, the Centers for Disease Control and Prevention issued a list of 12 Asian countries that are at high risk of a ZIKV epidemic, and Pakistan is among them. In 2016, a qualitative study was conducted to determine the potential risk of ZIKV in Pakistan by visualizing incoming air traffic from countries with ZIKV activity listed by the US Centers for Disease Control and Prevention. They revealed the transmission of ZIKV in Pakistan through travelers from Miami, Singapore, Florida, other parts of the United States, and Brazil (Butt et al. 2016).

### Future perspectives

Our recent study identified the strong association between MBDs and risk factors. Apart from this, we should follow and improve public health measures, such as the development and upgradation of labs for screening of these viruses to acquire more entomological surveillance data, real-time disease monitoring and strict vigilance at the border area, and public awareness regarding vector control and disease.

Given the high incidence of dengue, ongoing WNV transmission, and limited JE incidence, patients with acute encephalitis should be tested using nucleic acid-based diagnostic testing, which has greater specificity against *flaviviruses*.



Pakistan lacks a competitive nationwide surveillance system for arbovirus infections. Therefore, there are limited data regarding the types of circulating vectors, temporal trends, and their correlations with climatic factors and human/animal cases. In light of the heightened alert of epidemic outbreaks and the global spread of arboviruses such as WNV, JEV, CHIKV and ZIKV, there is a desperate need for active surveillance in Pakistan for the effective implementation of preventive control strategies.

Moreover, the importation of goods either from highways or shipping ports from disease-affected countries is an important source of disease spread. Recently, Pakistan has faced an active circulation of Crimean-Congo hemorrhagic fever, CHIKV and WNV that affects economic and social aspects of human lives. International collaboration with developed countries where these infections are going to be overcome is essential regarding the development of policies for effective control measures.

To improve the health care system in Pakistan, we should promote the One Health approach by inviting all concerned professionals, such as physicians, veterinarians, environmentalists, concerned authorities, and educated civilians, to play a vital role in preventing the epidemic of viral diseases by assessing and mitigating risk factors. Identification of research gaps, promotion of applied research, design of massive public awareness programs, training of intellectual staff, and personal assistance for capacity building are indispensable measures to tackle inadequacies.

## Conclusion

In Pakistan, the diagnosis of these mosquito-borne viruses is a great challenge, and well-equipped infrastructure is deficient in many labs. Clinicians are only inclined toward dengue infection diagnosis due to its endemicity from the last 10-12 years, and yet, they do not think about other arboviral infections, such as CHIKV, WNV, JEV, and ZIKV. Lack of initial screening kits at secondary health care levels leads to misdiagnosis of infections. This is due to the same clinical manifestations shared by these MBDs. In developing countries such as Pakistan, epidemiological studies are usually based on IgM positivity. However, in these mosquito-borne viruses, IgM cross-positivity was also observed, and conflicts with neutralizing antibodies were observed, especially in the case of DENV, WNV, JEV, and ZIKV. These issues raise questions regarding the true prevalence of these arboviral diseases in Pakistan. There is no approved vaccine for the control of these diseases in Pakistan, and the country relies solely on vector control. The health care sector must devise a strategy to control these outbreaks throughout Pakistan.

## Abbreviations

CHIKV: Chikungunya virus; DENV: Dengue virus; ELISA: Enzyme-linked immunosorbent assay; GBS: Guillain-Barré Syndrome; Ig: Immunoglobulin; JE: Japanese encephalitis; JEV: Japanese encephalitis virus; ZIKV: Zika virus; MBDs: Mosquito-borne diseases; NIH: National Institute of Health; WHO: World Health Organization; WNV: West Nile virus

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## Authors' contributions

MI, JY and SC wrote the manuscript. MI, IS and MKS collected data. MI and AZ prepared the figures. ZC, JY and SC provided critical feedback and helped with manuscript analysis. All authors read and approved the final manuscript.

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## Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

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### Competing interests

Authors Huanchun Chen and Shenbo Cao have not involved in the journal's review or decisions related to this manuscript. The authors declare that they have no competing interests.

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