INTRODUCTION

Dengue is currently considered as one of the most significant arboviral diseases of humans worldwide, predominantly distributed in tropical and subtropical regions that are the natural home for its transmission vectors, i.e., mosquitoes of the genus *Aedes*. The causative aetiological agent is the *Flavivirus* genus of family *Flaviviridae*, otherwise called dengue virus (DENV). This family is closely related to viruses which cause other notable infectious diseases in humans including yellow fever, West Nile encephalitis, Japanese encephalitis and hepatitis C virus infection. There are now five recognised serotypes, DENV1-5, characterized by neutralization assays. Each enveloped virion contains a single-stranded RNA genome of positive polarity. This is a single open reading frame 10.7 kilobases in length that encodes a precursor polypeptide for three structural proteins, capsid (C), pre-membrane (prM) and envelop (E), and seven non-structural (NS) proteins. Both 5' and 3' ends are flanked by an untranslated region (UTR), making the overall genome structure 5'-UTR-C-prM-E-NS1-NS2a-NS2b-NS3-NS4a-NS4b-NS5-UTR-3'. While humans are the primary host DENV also infects non-human primates such as macaques. The virus is transmitted by female *Aedes* mosquitoes, particularly *Ae. aegypti* and *Ae. albopictus*. Infection with DENV may be subclinical or symptomatic. Clinical illness is traditionally classified, in order of increasing severity, as either dengue fever (DF), dengue haemorrhagic fever (DHF) or dengue shock syndrome (DSS). More recently, the WHO proposed a revised classification of clinical infection: dengue; dengue with warning signs; and severe dengue. DF is due to primary infection with any of the serotypes and is typically mild and self-limiting. Recovery from infection is generally complete and confers lifelong homotypic immunity. DF manifests as a fever for 2–10 days, headache, retroorbital pain, joint and muscle pain with skin rashes. Secondary infection with a heterotypic serotype generates cross-reactive antibodies, the presence of which increases...
the potential risk of antibody-dependent enhancement of disease, a form of immunopathology. Hence, recurrent infection is the major risk factor for the serious, often fatal, complications of DHF and the rarer DSS. These are marked by problems of capillary permeability, a decrease in platelet count, disordered blood clotting and severe bleeding, and for DSS alongside systemic shock leading to organ failure. Different dengue serotypes vary in their capacity to cause severe illness, but there is no clear consensus on a causal association. At present, there is neither specific anti-dengue therapy nor a preventive vaccine available commercially to combat this globally expanding public health problem. The existence of multiple DENV serotypes in the same locality is a major threat to resident communities.

Global emergence of dengue

The recorded history of dengue in humans is very long. What appear to be dengue-like symptoms were described in a Chinese medical encyclopaedia written over 1000 years ago. However, there is no uniform agreement on the geographical origin of DENV. Evidence indicates the first noted presence of mosquito viruses in forests of Asia and/or Africa, with major epidemics in humans occurring in Asia, Africa and North America during the 1780s. The rapid spread of dengue all across the world can be related to the global dissemination of its principal vector. Deforestation in order to provide new environments for human residence commensurate with population growth has inadvertently enforced cohabitation with DENV, in novel environments where the peridomestic mosquitoes, Ae. aegypti and Ae. albopictus facilitate transmission. The disease started to exert a major impact globally in the early and mid-20th century, advanced by a combination of human activities like the slave trade, migration, inter-country and -continental commerce, and two devastating world wars. As a result, there has been a >30-fold escalation in dengue incidence worldwide between 1960 and 2010. Uncontrolled population growth, unplanned urbanization, increased air travel, insufficient public health care facilities, ineffective vector control and global warming are some of the important factors that promote the blooming of dengue, making it a global pandemic threat. Currently, a total population of 3.6 billion people in over 125 countries are considered at risk. This leads to some 50–100 million clinical cases annually. Of these, most people recover without ongoing problems but 2.5–5% requires hospitalization for life-threatening complications. A high mortality rate in children contributes to around 25,000 deaths each year.

Southeast Asian countries are particularly severely affected by dengue epidemics. Many cases of dengue infection occurred in the Philippines and Thailand during the 1950s. Over the following two decades, transmission dispersed so widely that today DHF is a leading cause of morbidity and mortality among children across the region, notably India. Furthermore, dengue incidence increased markedly in the Western Pacific Islands over the same time. At the start of this century, the disease emerged in Bangladesh, Bhutan and Nepal. Since then, the dengue epidemic has intensified all over the world. The WHO reported that since 1960, every 10 yr the average annual number of cases of DF/DHF has grown exponentially.

Emergence of dengue in Nepal

Nepal is a landlocked developing country located in the central Himalayas region of South Asia. Geographically, it has three distinct ecological zones: The Northern Range – Mountain; the Mid Range – Hill region; and the Southern Range – Terai (“flat land”) (Fig. 1). The country has an extreme topography and climate. The altitude ranges from 70 m above sea level, on the southern border with India to 8848 m, the highest point on Earth, in the mountains bordering China. Consequently, the climate varies from tropical to arctic depending on the altitude. Just over 50% of Nepal’s current population (of nearly 28 million) resides either in a tropical or in a subtropical climate, where almost all dengue outbreaks have occurred to date. Given the annual growth rate of 1.35%, millions more individuals are set to be exposed to the threat of dengue infection in coming years.

Although, dengue is well-established in neighbouring India, with which borders are open, for reasons that are not apparent, Nepal was dengue-free, or at least was considered as such, until the first recognised suspected incidence in 2004. This case was that of a Japanese visiting worker who stayed in Nepal for several months and tested seropositive for the disease on return to his home country. This first Nepalese isolate was identified by genomic analysis as DENV-2 (GenBank accession no. AB194882), which showed 98% genomic similarity with DENV-2 isolates from India, from which the reasonable conclusion was drawn that it originated from India and passed into Nepal via another previously infected – and undiagnosed – individual, or less likely, through translocation of an infectious mosquito. While this marks the official start of the story of dengue in Nepal, it is probable that infections went undiagnosed before this time due to a lack of requests for testing by unsuspecting
clinicians combined with limited medical microbiology diagnostic laboratory facilities in most provincial areas. A counterpoint to this, however, is provided by a study conducted in 2001 involving 876 patients from the capital city of Kathmandu, located in the Mid Range, which did not detect dengue-specific immunoglobulin (Ig)M antibodies, indicative of prior infection, in any individual’s blood. Following the first case report just over a decade ago, several studies have been conducted to determine the prevalence of dengue infection in contrasting regions of Nepal. Of screened sera from 103 patients presenting with acute fever in Kathmandu eight individuals tested positive for dengue-specific IgM. Hence, transmission of dengue in Kathmandu and the surrounding Hill region was confirmed by this report published in 2007. Nevertheless, dengue is considered more common in the lower Terai region. A study which included both Terai and Hill regions showed that 80% of DENV-positive serum samples came from persons living in the Terai. The same study confirmed the presence of all four then known serotypes of DENV in Nepal. Relatively fewer patients reported previous travel to a dengue-endemic region of neighbouring India, the majority having a very limited travel history. This is strongly indicative of local transmission of dengue infection in different areas within Nepal. Moreover, entomological investigation identified both major dengue vectors, *Ae. aegypti* and *Ae. albopictus*, in the same locations.

Similarly, in 2006 the incidence of dengue rose in the southwest region of Nepal, where a prevalence rate of 10.4% for anti-DENV IgM was noted among patients with pyrexic illness. Both DF and DHF were diagnosed clinically and serologically. However, this was not confirmed by virus isolation and molecular analysis since the required medical microbiology facilities were not available. Interestingly, almost all patients had no history of travel to India or other dengue-endemic areas. This would appear to indicate that DENV had been circulating locally for a substantial period prior to 2006. Hence, in Nepal dengue infection was likely to have been misdiagnosed prior to this time and thus illness caused by DENV was either under reported or, before 2004, not recognised at all. Nepal has no dengue surveillance programmes, and health professionals do not usually consider dengue as a possibility when making a differential diagnosis.

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**Fig. 1:** Map of Nepal showing topography, political boundaries and access routes—Modified from publication of Office for the Coordination of Humanitarian Affairs (OCHA) United Nations, Nepal, August 2005.
diagnosis\textsuperscript{28}. In a subsequent study in the Terai, the prevalence rate was, at 30\%, much higher still\textsuperscript{29}. Of note, dengue was detected in the eastern part of Nepal, where the incidence was in fact higher than that observed in central Terai, which is considered to be a high risk zone\textsuperscript{30}. A very similar anti-DENV IgM positivity rate of 29.3\% was reported for a study undertaken in 2007–08 in western Terai, with the highest number of cases following the rainy season in October\textsuperscript{31}.

In contrast, a cross-sectional analysis of the same area in the following year (2009) revealed that seropositivity was only 9.8\%\textsuperscript{32}. Diagnosis of dengue was highest in Mahendra Nagar (13.3\%) followed by Dhangadhi (9.8\%); both cities in the far west of Nepal and very close to India. In an independent descriptive cross-sectional study carried out in several geographically diverse regions of Nepal from June to September 2009, overall anti-dengue IgM positivity was 12.17\% with the greatest prevalence in the far west district of Kanchanpur (capital city Mahendra Nagar, now called Bhimdatta), followed by the provincially quite separate south central Chitwan\textsuperscript{33}. For Bharatpur, the capital city of this latter district and the fifth largest metropolitan area in Nepal, an independent study conducted from January 2010 to December 2011 reported an overall seropositivity of 8.5\% among clinically suspected patients presenting at a tertiary care centre; and during December 2010, when the most number of cases were reported, this rate rose to 90.4\%\textsuperscript{34}. This constituted part of the largest outbreak of dengue in Nepal to date (Table 1).

The 2010 epidemic started at the end of August 2010 and spread rapidly throughout the country’s central region. In concurrence with the Bharatpur reporting, the hardest hit district was Chitwan, where 24 people died of dengue and > 7000 were found to be experiencing clinical manifestations of disease\textsuperscript{35}. However, governmental statistics reported fewer cases\textsuperscript{36}, since only data from governmental health care facilities were included. It is acknowledged that there has been significant underreporting of cases in Nepal due to a combination of few referrals to specialist infectious disease physicians, inadequate number and quality of diagnostic laboratory facilities, insufficient research on vector bionomics and disease epidemiology, and a lack of virological surveillance\textsuperscript{37}. DENV-1 was found responsible for the 2010 epidemic and the implicated strain is phylogenetically close to the predominant Indian strain\textsuperscript{38}. However, this study did not indicate the presence of other DENV serotypes, all of which have been identified previously in Nepal\textsuperscript{28}. Entomological investigation identified both Ae. aegypti and Ae. albopictus in 2010 epidemic areas. This outbreak is thought to have originated in the Terai region and expanded into the Hill areas\textsuperscript{37}. A more recent report points out that dengue vectors are well established in tropical and subtropical regions of Nepal\textsuperscript{39}. Another related study by the same authors shows that knowledge of dengue fever – what it is, how it spreads and how it can be controlled – among Nepalese people is disconcertingly low\textsuperscript{40}.

All of these reports confirm Nepal as a dengue-endemic country with over half of the current population living in regions where they are at risk of infection. The major factors associated with the emergence and rapid escalation of clinical cases of dengue in Nepal are yet to be analysed. However, the most probable causes include an increase in vectors, both in terms of total numbers of mosquitoes and their geographical range, facilitated by expansion of suitable environments for their transmission as a consequence, direct or indirect, of global warming. Given the febrile nature of dengue, the undifferentiated and non-specific disease symptoms overlap with those caused by other viral infections, notably Japanese encephalitis with which dengue co-circulates in some parts of the country\textsuperscript{41}. Thus, it is not surprising that while dengue seropositivity is detected in increasing numbers, at this time it is not responsible for the greater proportion of illnesses presenting initially as fevers of unknown origin. However, the remarkable expansion of dengue in Nepal means that the upward trend is set to continue\textsuperscript{40}. From the perspective of public health management this is a double-edged sword since the occurrence of more clinical cases will lead to greater recognition among public health workers, a call for improved diagnosis\textsuperscript{42}, and, ultimately, to increased public awareness, which is required for implementation of effective preventive measures. A key question to raise is what, in terms of present and predicted cases of dengue, is considered the tipping point for consolidated, coordinated action.

\textit{Increase in geographical range of vectors}

DENV-transmitting mosquitoes, primarily Ae. \textit{aegypti}
Aedes aegypti and Ae. albopictus, are found throughout tropical and subtropical regions of the world, but most frequently between latitudes 35°N and 35°S where the winter isotherm does not fall below 10°C. These day-biting mosquitoes are most active for the period between two hours after sunrise and several hours before sunset. Despite both now being present worldwide in tropical and subtropical regions, each species of Aedes has a distinct ecological habitat. Ae. aegypti originated in Africa but is extending increasingly into temperate zones during both the northern and southern hemisphere summer months. Commonly known as the yellow fever mosquito, Ae. aegypti feeds preferentially on humans, lives in or near residential dwellings, and lays its eggs in artificial water containers in and around houses. Ae. albopictus originated in Southeast Asia (common name—Asian tiger mosquito), but its range is now expanding globally. Females feed on blood from not just humans but other mammals and sometimes birds, and lay eggs on the inside of water-holding vessels as well as near water on the edge of forests (hence, also known with its alternative name of the forest mosquito). Both species have a limited flight capacity and individuals spend their time in the close vicinity of the houses where they emerge as adult. The noticeable broadening of the range of Aedes in recent times is attributable to inadvertent dissemination of vectors by transport of goods and people, particularly via international air travel, then increased survival in its new location, especially in summer months. Together, this enables a foothold in the translocated environment, hence facilitating the wider geographical transmission of dengue.

Since, very few studies of dengue vectors in Nepal have been performed, it is difficult to conclude when they started to appear. There was no evidence of the existence of Ae. aegypti until detected in Kathmandu district in 2009. However, Ae. albopictus was reported in Nepal dating back to 1956. In the same series of studies, several other species of Aedes were identified in different regions but this did not include Ae. aegypti. It is thought that this mosquito might have been particularly susceptible to the harsh winter climate. Even now, due to lower temperatures, Ae. aegypti is relatively uncommon in areas above 1000 m. The factor that is associated the most with the recent blooming of these vectors is global warming. As temperature increases, the mosquito population grows substantially. Data from around the world indicate that increasing dengue transmission correlates directly with a rise in temperature. However, seasonal transmission cycles in tropical areas almost always occur during the cooler rainy season, indicating that an elevation of temperature is not solely responsible for the observed increase in incidence of dengue—other factors must also play a role.

**Urbanization**

The population of non-rural areas of Nepal has increased rapidly over the last few years, resulting in unplanned and poorly controlled urbanization. Substandard quality of housing, overcrowding, and deterioration in water treatment, sewer and waste management systems are factors related to haphazard urbanization that together have created ideal conditions for increased transmission of mosquito-borne diseases in fast-growing cities in tropical regions. According to the United Nations report urbanization has taken place rapidly in Nepal in recent decades. In this context of socioeconomic deprivation, gradually rising average annual temperatures and the increased precipitation that comes with these facilitates rich breeding grounds for mosquitoes.

Unplanned, rapid urbanization can lead to dramatic changes in dengue transmission since this promotes increased exposure of the human population to the Aedes vector. In poorer localities, there is a pressing need to store water in cisterns and rain barrels in order to safeguard a regular, adequate water supply throughout the year. Poorly maintained sanitation and refuse services, as well as uncovered, damaged or leaking water vessels produce ready-made breeding sites for these anthropophilic mosquitoes.

**CONCLUSION**

Held on record in hospitals across Nepal, there are a large number of formal and informal clinical case reports of persons presenting with a fever of unknown origin, many of which went undiagnosed. Purely on a statistical basis, it can be reasoned that many of these were dengue cases. In spite of limited investigation of this viral infection in the country, what records are available until now are sufficient to conclude that this Himalayan nation is at high risk of future dengue epidemics. Furthermore, co-circulation of multiple serotypes has the potential to worsen any outbreak by increasing significantly the risk of a person contracting recurrent infection and hence suffering the immunopathology-driven complications of severe dengue. The increasing trend for migration of people from rural to urban areas, where the mosquito vector is more commonly found, means that a growing proportion of the population will be under threat of infection in future. In order to address this major public health issue in Nepal, several factors that contribute to the present inadequate implementation of an anti-dengue public health...
strategy require improvement. These include a paucity of funding for medical treatment, poor facilities for disease diagnosis, a scarcity of mosquito control programs and inadequate entomological surveillance. A concerted action plan that focuses on these issues would help to not only trace the source of any outbreak but to alleviate the current and predicted escalating burden of morbidity and mortality imposed by dengue in Nepal.

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Conflict of interest

The authors declare that they have no conflict of interest.

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