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International Journal of Applied Sciences and Biotechnology

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EMERGENCE OF DENGUE VIRUS INFECTION IN NEPAL

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Abstract

This article reviews Dengue, a common viral disease in humans and is an emerging public health problem in Terai Region of Nepal. The most affected are among the poorest populations living in remote, rural areas and urban slums who have even no access for medical treatment, acquired by bite of infected mosquito. *Aedes aegypti* infected with dengue virus is the major source of infections for humans and cannot be transmitted from person-to-person because human are the dead end host. DENV-1 was first isolated by Ren Kimura and Susumu Hotta in Japan in 1943. An epidemic of DF involving at least 200,000 cases had occurred between 1942 and 1944 during World War II in Japanese port cities such as Nagasaki, Kobe, and Osaka. DF/DHF is now endemic in more than 100 countries and threatens the health of about 40% of the world's population (2.5 billion), particularly in tropical and subtropical regions and predominantly in urban and periurban areas. Over 1.2 million cases were reported to WHO in 1998, the greatest number ever for a single year. There are an estimated 50 million infections annually, including 400,000 cases of DHF (WHO, 1999). Dengue is a global health problem and its expanding endemicity towards new territories is a serious concern. Relatively a new disease in Nepalese context, first case of dengue was reported in 2004 in Nepal. There was an epidemic in 2006 then Dengue abruptly appeared as massive outbreak in 2010, merely six years after its first introduction in 2010 with death of 20 people in central region of Nepal i.e. Chitwan. The weight of the evidence suggests that the re-emergence of dengue in Nepal resulted from the introduction of the infection by travellers from India where dengue is endemic.

Keywords: Dengue virus, Epidemic, IgM ELISA, *Aedes aegypti*

Introduction

Dengue viruses (DENVs), which belong to the genus *Flavivirus*, family *Flaviviridae*, comprise four serotype named dengue virus types 1, 2, 3, and 4 (DENV-1, -2, -3, and -4). Infection with any of these serotypes leads to a broad clinical spectrum, ranging from sub-clinical infection or an influenza-like disease known as dengue fever (DF) to a severe, sometimes fatal disease characterized by haemorrhage and shock, known as dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS). DENVs are transmitted to humans mainly by the bites of *Aedes aegypti* and *Aedes albopictus* mosquitoes. Dengue is the most important arthropod-borne virus in tropical and subtropical countries, with an estimated 50 million infections each year, resulting in 500,000 cases of DHF/DSS and 25,000 deaths.

Like other flaviviruses, dengue virus has a single-stranded, Positive - sense RNA genome of ~10,700 nucleotides, surrounded by a nucleocapsid and covered

by a lipid envelope that contains the viral glycoproteins. The RNA genome contains a single open reading frame (ORF) flanked by two untranslated regions (5' and 3'UTRs). The single ORF encodes a precursor polyprotein, which is co- and post-translationally cleaved resulting in the formation of three structural proteins, Capsid (C), membrane (M), and envelope (E), and seven non-structural proteins, NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5. There is no specific antiviral therapy or vaccine in clinical use for dengue fever. Medical care is supportive in nature and focuses on monitoring and administration of fluids to prevent dehydration and shock, medications to lower fever and reduce pain, and management of bleeding complications.

Global scenario of Dengue virus infection

DENV-1 was first isolated by Ren Kimura and Susumu Hotta in Japan in 1943 (Kimura and Hotta, 1943). An epidemic of DF involving at least 2,00,000 cases had

occurred between 1942 and 1944 during World War II in Japanese port cities such as Nagasaki, Kobe, and Osaka. The infections originated from persons returning from the tropics, in particular Southeast Asia and the Pacific islands (Hotta *et al.*, 2000). A few months after the first isolation of DENV-1 in Japan, Albert Bruce Sabin and Walter Schlesinger isolated DENV-1 from Hawaiian and shortly thereafter, DENV-2 from Papua New Guinean samples (Sabin and Schlesinger, 1945). They demonstrated that these viruses were antigenically related, yet distinct, and they could be distinguished by the hemagglutination inhibition (HI) assay. In the late 1960s, DHF fatality has been reported to be as high as 41.3% (Sumarmo *et al.*, 1987) when healthcare providers understandably were still unfamiliar with the disease. Today, DHF fatality rates can exceed 20% without proper treatment, but can be brought down to 1% with proper medical care (WHO, 1999). Although there were various speculations about the earliest description of *dengue-like* diseases in historical accounts (Halstead, 1980 *et al.*, 1980) the disease now known as DHF was first recognised in Manila, the Philippines in 1953 (Quinlos *et al.*, 1954). Viruses similar to DENV-1 and DENV-2 were isolated from Manila patients in 1956 by William Hammond and were called DENV-3 and DENV-4. Dengue viruses of multiple serotypes were subsequently isolated from patients of another DHF epidemic in Bangkok, Thailand in 1958 (Hammond *et al.* 1960). It is now known all four serotypes of dengue virus can cause DHF. DHF/DSS outbreaks were mainly restricted to Southeast Asia until the early 1980s (Halstead, 1980). Since then, dengue transmission has intensified and DHF/DSS outbreaks are now frequent in most tropical countries. To this day, DHF/DSS remains a leading cause of hospitalisation and death among children in Southeast Asia. Outside the region, the disease burden of dengue is most acutely felt in Central and South America where 24 countries have reported laboratory-confirmed DHF between 1981 and 1997 (Monath *et al.* 1994).

DENV-1 can be divided into five genotypes based on the complete E gene sequence as described by Goncalvez *et al.* (2002). Earlier work by Rico-Hesse (1990) also classified DENV-1 into five groups based on the 240- nucleotide E/NS1 junction sequence. The DENV-1 genotypes all have a wide area of distribution apart from genotype III (sylvatic) and genotype II which consists of Thai strains from the 1950s and 1960s. Viruses of genotype I and IV have recently been implicated as causing epidemics in the Pacific between 2000 and 2004 and genotype V viruses are frequently

isolated during epidemics in the Americas. However, it is still inconclusive whether any of these three DENV-1 genotypes can be consistently associated with causing more severe dengue.

Table 1: DENV-1 genotypes according to Goncalvez *et al.* (2002)

Genotypes	Original known distribution
I	Japan, Hawaii in the 1940s (the prototype strains), China, Taiwan and Southeast Asia
II	Thailand in the 1950s and 1960s.
III	Sylvatic source in Malaysia.
IV	Nauru, Australia, Indonesia and the Philippines.
V	Africa, Southeast Asia and the Americas.

DENV-2 is the most studied serotype among the dengue viruses. Twiddy *et al.* (2002) proposed the existence of six genotypes of DENV-2 (Table 2) based on the complete E gene sequence following earlier work by Rico-Hesse (1990) and Lewis *et al.* (1993). Sylvatic DENV-2 strains that are closely related have been isolated from several countries in West Africa and Malaysia, two locations that are far apart, leading to hypothesise that the DENV sylvatic ancestor arose in the Asian-Oceanic region before diverging into today's four DENV serotypes. The first DHF epidemic in the Americas occurred after an introduction of the Asian II genotype to Cuba in 1981 (Guzman *et al.*, 1995). Likewise, the America/Asian genotype (genotype III) has been reported to have replaced the pre-existing American genotype (genotype V) in the Western Hemisphere and is considered to be the DENV-2 genotype with the highest epidemiological impact.

The current genotype classification for DENV-3 follows the nomenclature proposed by Lanciotti *et al.* (1994) which recognised four DENV-3 genotypes based on prM/E sequences (Table 3). These four genotypes are similar to the four groups using a 195-nucleotide region at the 5' terminus of the E gene. Introduced to the Americas via Nicaragua in 1994, genotype III DENV-3 is now widely found in Central and Southern America (Balmaseda *et al.*, 1999; Usuku

et al., 2001; Messer et al., 2003) and is considered as the most virulent of the four DENV-3 genotypes. It is worthy of note that genotype IV has never been associated with any DHF epidemics. Although their existence is anticipated through the presence of DENV-3 antibodies in non-human canopy-dwelling primates, no sylvatic lineage of DENV-3 has been found thus far (Rudnick, 1984).

Table 2: DENV-2 genotypes according to Twiddy et al. (2002).

Genotypes	Original known distribution
American	Formerly known as subtype V. Found in Latin America, old strains from India (1957), the Caribbean, and the Pacific islands between 1950 and 1970s.
American/Asian	Formerly known as subtype III. Found in China, Vietnam, Thailand and in Latin America since the 1980s.
Asian I	Thailand, Myanmar and Malaysia.
Asian II	Formerly known as subtype I and II. Found in China, the Philippines, Sri Lanka, Taiwan and Vietnam. Includes the New Guinea C prototype strain.
Cosmopolitan	Formerly known as genotype IV. Wide distribution including Australia, the Pacific islands, Southeast Asia, the Indian subcontinent, Indian Ocean islands, Middle East, and both East and West Africa.
Sylvatic	Isolated from non-human primates in West Africa and Malaysia.

Table 3: DENV-3 genotypes according to Lanciotti et al. (1994) and the known distribution of the genotypes prior to 1993.

Genotypes	Original known distribution
I	Indonesia, Malaysia, Thailand, Burma, Vietnam, the Philippines and the South Pacific islands (French Polynesia, Fiji and New Caledonia). Includes the H87 prototype strain.
II	Thailand, Vietnam and Bangladesh.
III	Singapore, Indonesia, South Pacific islands, Sri Lanka, India, Africa and Samoa.
IV	Puerto Rico and French Polynesia (Tahiti).

Lanciotti et al. (1997) initially separated DENV-4 into two genotypes, I and II, based on the complete E gene sequence. A further two genotypes were subsequently described (Table 4), with one found only in non-human primates in Malaysia and another, genotype III, found only in Bangkok, Thailand (Klungthong et al., 2004). Genotype II DENV-4 is the most widespread of the four following an introduction to the Western hemisphere in 1981, possibly via the Pacific islands (Lanciotti et al., 1997; Foster et al., 2003). Although DENV-4 is the least frequently sampled serotype, it is often associated with haemorrhagic fever during secondary infection (Vaughn et al., 2000).

Table 4: DENV-4 genotypes and their known distribution

Genotypes	Original known distribution
I	Thailand, Malaysia, the Philippines and Sri Lanka. Includes the H241 prototype strain
II	Indonesia, Malaysia, Tahiti, the Caribbean islands (Puerto Rico and Dominica) and the America.
III	Thailand (Bangkok, specifically).
Sylvatic	Isolated from non-human primates in Malaysia.

Scenario of Dengue virus infection in South East Asia Region

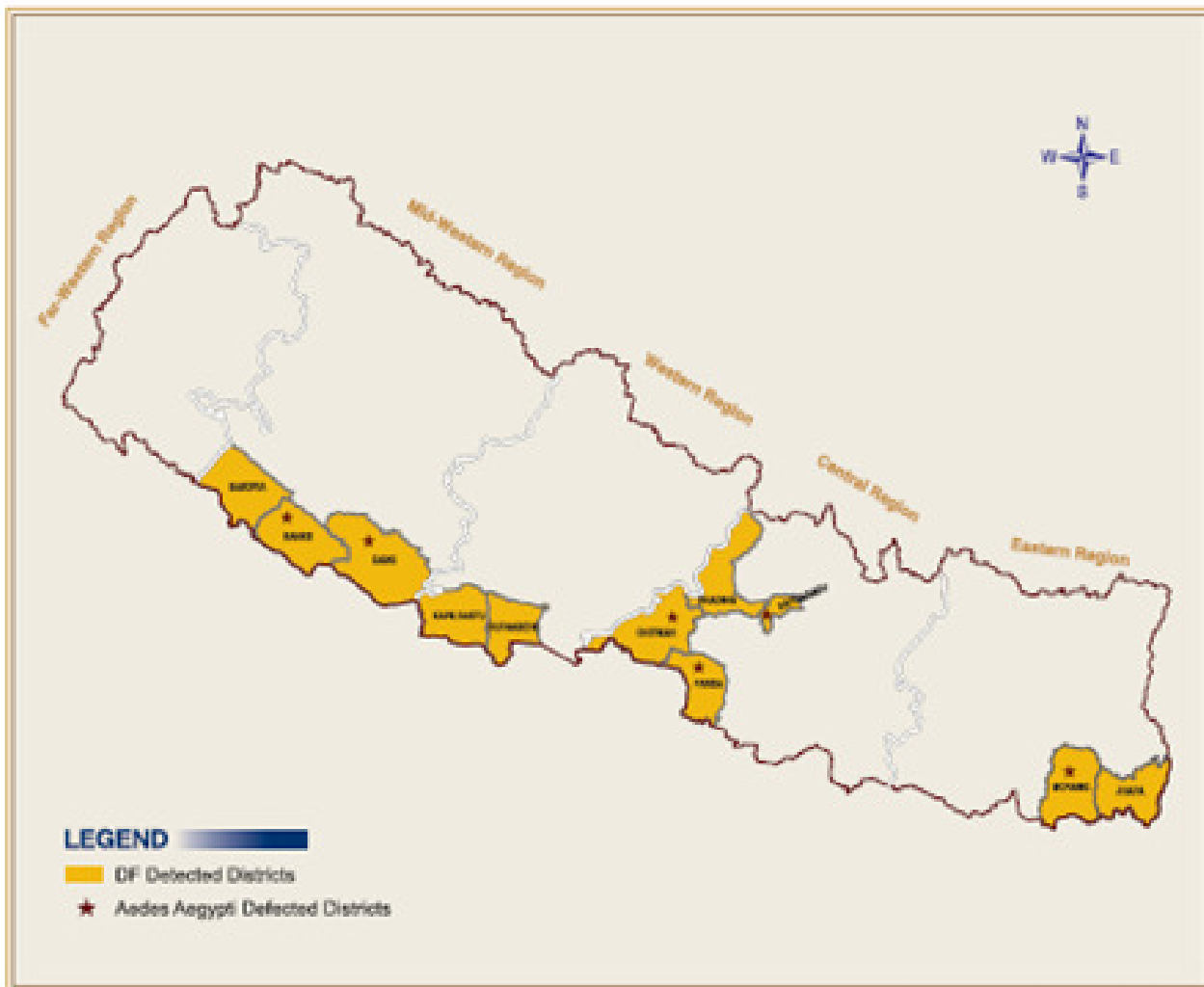
Of the total world population of 6.2 billion, countries of the South-East Asia Region (SEAR) account for 1.5 billion (24%). On that scale, of the 2.5 billion people (living in the tropics and sub-tropics) at risk of DF/DHF, 52%, i.e. 1.3 billion populations, live in SEAR. In 2003, only 8 countries in South East Asia

Region reported dengue cases. As of 2006, ten out of the eleven countries in the Region (Bangladesh, Bhutan, India, Indonesia, Maldives, Myanmar, Nepal, Sri Lanka, Thailand and Timor-Leste) reported dengue cases. Bhutan reported the first dengue outbreak in 2004. An outbreak, with a high case fatality rate (3.55%) was first reported in Timor -Leste in 2005. Nepal reported dengue cases for the first time in 2004 from Chitwan district. The Democratic Peoples' **Nepalese scenario of Dengue virus infection**

DF is an emerging disease affecting Nepal since 2004 (Pandey *et al.*, 2004) There was an concern of the disease in Nepal after the Indian and Pakistani epidemic of DF/DHF, which claimed more than 100 deaths and more increasingly several thousand cases in the year 2006 (Gupta *et al.*, 2006) There was an outbreak which was observed in 9 districts of Tarai region in Nepal in 2006 (Pandey *et al.*, 2008). In

Republic of Korea is the only country in this Region of WHO that has no report of indigenous transmission of DF/DHF. For the South East Asia Region as a whole, there is about 18% increase in number of reported cases and about 15% increase in the number of reported dengue deaths in 2007 as compared to same period last year. There was substantial increase in the reported cases of dengue in Thailand, Indonesia and Myanmar.

of DF in Banke, Bardiya, Dang, Kapilbastu, Parsa, Rupandehi, and Jhapa districts. A total of 70 serum samples from suspected DF cases were collected from 19 districts. So far, 22 cases of DF had laboratory confirmed and many patients had travel history to India. It was also reported that many patients having similar symptoms visited India for treatment and confirmed as DF. Seventy-five per cent DF cases were reported in October and few cases were reported in September and November. Only 11 per cent patients



2006, there were reports of suspected DF outbreaks in Banke district. The clinical observation, pathological and laboratory investigation results proved introduction

had travel history to India in past two week period prior to clinical manifestation of DF. Ninety-four per cent patients were adults and male to female ratio was 4:1

under reporting is expected in the absence of diagnostic facilities at the field level and it may be reported either as viral fever or Pyrexia of Unknown Origin (PUO). Over dependency on single serological test (mostly rapid) has led to underestimation of dengue burden in the country. However, there had been a major outbreak of Dengue Fever in central Nepal starting from the September 2010 in Chitwan and adjacent districts of Nepal. Around 24 people have died of dengue fever and more than 7000 are thought to have contracted it during the this outbreak. In the past two months cases have also been diagnosed in other central districts and in the west of the country. Moreover, co-circulation of all serotypes possesses a threat for severe dengue outbreaks in the future. Due to the lack of dengue sequence data from Nepal, the exact evolutionary dynamics and origin of the virus in the country is not known. For better understanding of molecular epidemiology and origin of Dengue virus infection in Nepal we need to generate nucleotide sequence database. India may be the origin of Nepalese dengue virus strains based on the close genetic similarity observed and extensive cross-border activities

Outcome of Dengue Virus Infection

A person could suffer from dengue infection four times throughout his/her lifetime, once for each of the four DENV serotypes. Both primary (first) and secondary (subsequent) infections with any serotype of DENV can result in either the clinically less severe DF or the more severe DHF (Rosen *et al*, 1977). A primary dengue infection confers the recovered patient life-long immunity against the infecting serotype and a brief protection against infection by other DENV serotypes (Sabin *et al*, 1952). However, epidemiological data and some studies suggest that the immunity thus gained, after the lapse of the temporary cross-serotypic protection, increases the probability of an individual developing DHF when infected by a second heterologous DENV serotype Halstead *et al.*, 1967; Halstead *et al.*, 1970). A hypothesis to explain this phenomenon, called antibody-dependent enhancement (ADE), proposes that pre-existing sub-neutralizing antibodies from the primary infection and the second infecting DENV serotype form complexes that bind to cells bearing Fcγ receptor (FcγR) (monocytes and B cells) leading to increased virus uptake and replication. (Halstead *et al*, 1988).

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