# Dengue Virus Serotype 2 from a Sylvatic Lineage Isolated from a Patient with Dengue Hemorrhagic Fever

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## Abstract

Dengue viruses circulate in both human and sylvatic cycles. Although dengue viruses (DENV) infecting humans can cause major epidemics and severe disease, relatively little is known about the epidemiology and etiology of sylvatic dengue viruses. A 20-year-old male developed dengue hemorrhagic fever (DHF) with thrombocytopenia (12,000/ul) and a raised hematocrit (29.5% above baseline) in January 2008 in Malaysia. Dengue virus serotype 2 was isolated from his blood on day 4 of fever. A phylogenetic analysis of the complete genome sequence revealed that this virus was a member of a sylvatic lineage of DENV-2 and most closely related to a virus isolated from a sentinel monkey in Malaysia in 1970. This is the first identification of a sylvatic DENV circulating in Asia since 1975.

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# Introduction

Dengue viruses cause dengue fever (DF) and dengue hemorrhagic fever (DHF) in the tropics from Southeast and Southern Asia, the Caribbean, and many countries in South and Central America, and outbreaks are reported with increasing frequency globally. The four distinct dengue virus serotypes (DENV-1 to DENV-4) belong to the family *Flaviviridae* and are among the most important vector-borne pathogens of humans, causing up to 100 million cases annually.

Dengue viruses are mosquito-transmitted and circulate in both a sylvatic (enzootic) cycle involving non-human primates and various species of Aedes mosquito (such as Ae. furcifer, Ae. luteocephalus and Ae. taylori), and in a human (endemic) cycle principally vectored by Aedes aegypti [1,2,3]. The only sylvatic DENV serotype that has been isolated in Africa is DENV-2. In contrast, sylvatic DENV-1, DENV-2 and DENV-4 have been isolated in Asia [2], although the last isolation of a sylvatic virus (of DENV-4) occurred in 1975, and the 'sylvatic' isolate of DENV-1 is not phylogenetically distinct from human lineages so that its origin is uncertain. Phylogenetic data suggests that sylvatic DENV are the ancestors of those viruses that now circulate endemically in human populations [2]. Importantly, sylvatic dengue viruses have never been associated with major epidemics in humans [4]. Here we report a case of dengue hemorrhagic fever from whom we isolated a DENV-2 which was determined by phylogenetic analysis to be a sylvatic strain most closely related to a virus isolated by Rudnick from a sentinel monkey in peninsular Malaysia in 1970 [5].

### **Materials and Methods**

#### The context

Our laboratory provides virology support in the state of Sarawak, Malaysia, part of which involves the isolation of virus from the blood of patients suspected of having DF or DHF. This is conducted as part of routine investigations for fever, which includes ruling out both dengue and malaria. Two primary care clinics and the university health centre act as sentinel clinics for public health surveillance to detect dengue in the community. During this routine surveillance program we isolated a sylvatic virus from a dengue febrile patient.

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#### Ethics statement

Further investigation of the virus isolated was performed after consultation with the patient and he has provided written consent to allow publication of his case.

#### Virus isolation

Serum from the blood drawn at presentation was inoculated into C6/36 mosquito cells. When a cytopathic effect characteristic of DENV was noted, the culture was harvested and RNA extracted using the High Pure Nucleic Acid Extraction kit (Roche Diagnostics, Indianapolis, IN) and RT PCR performed to amplify a part of the NS5 gene using primers mFU1 and CFD2 as described by Chien and coworkers [6]. The amplicon was purified, sequenced and submitted to a BLAST search that returned a match to DENV-2. We then amplified the E/NS1 junction using primers AS2622DEN2 and S11871DEN2 [7] and used the