

PanBio Pty. Ltd., Brisbane, Australia). The same acute-phase serum was tested for flavivirus RNA by seminested reverse transcription–polymerase chain reaction (RT-PCR) by using flavivirus consensus primers PF1S and PF2R as previously described (1) in conjunction with the sense primer PF3S (GCIATHTGGTAYATGTG-GYT). Attempts to isolate viruses by using C6/36 and Vero cells were unsuccessful, which might be expected given the delay between the onset of symptoms and specimen collection.

Sequence analysis of the 163-bp (primers excluded) PCR product (GenBank accession no. AY862501) showed 89%–99.4% range of homology with 34 dengue 3 virus strains by using the BLAST nucleotide program. Similarities obtained with sequences of dengue virus 1, 2, and 4 were  $\leq 87\%$ . Phylogenetic analysis performed with the patient sequence together with homologous sequences from dengue viruses and other flaviviruses showed that it corresponded to dengue 3 virus species. RT-PCR amplification on the convalescent-phase serum was negative. Based on World Health Organization criteria, the patient was diagnosed with dengue fever (2). The patient's interview showed a previous dengue fever episode in Haiti in 1995 and a previous malaria episode in Burundi in 2002, but biologic confirmation was not available, and serum was not collected before this episode. Therefore, we could not determine definitively whether this patient experienced primary or secondary dengue. In light of virologic tests results, the diagnosis of secondary dengue infection was more likely (3).

A PubMed search using the keywords dengue, mixed infections, dual infections, simultaneous infections, and concurrent infections retrieved 14 references published since 1958. In most cases, concurrent infection was with 2 dengue virus strains from 2 different serotypes in a single patient (4,5). Only 6 published studies report-

ed concurrent infection with dengue virus and a bacterium (*Salmonella typhi*, *Shigella sonnei*, *Leptospira* spp.) (6–8) or with a virus such as Chikungunya virus (9).

To our knowledge, this is the first report of mixed dengue–parasite infection, dengue virus with *P. falciparum*. The authors previously questioned the accuracy of a serologic test to diagnosis dengue fever in patients experiencing malaria because reactivity was nonspecific on certain rapid serologic assays (10); however, serologic tests used in this study have demonstrated good specificity (10), and molecular tests are not prone to such specificity problems. Classifying this case as dengue hemorrhagic fever is questionable since some of the hemorrhagic signs may have been caused by acute malaria. In cases of concurrent infections involving a dengue virus, questions related to the influence of mixed infection on severity and prognosis are, therefore, impossible to address because of lack of information. Further investigations are required because this situation likely occurs frequently in nature, despite scant available data.

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## West Nile Virus Detection and Commercial Assays

**To the Editor:** Roehrig and colleagues described the long-term persistence of immunoglobulin (Ig) M antibody in patients with West Nile virus (WNV) infection, as tested using an in-house Centers for Disease Control and Prevention (CDC) enzyme immunoassay (EIA) (1). This result suggests that interpreting WNV IgM results in subsequent years would be difficult. With the commercial availability and widespread use of US Food and Drug Administration–

approved WNV IgM tests, we were concerned that this phenomenon might also occur with new tests. Thus in 2004, we initiated a follow-up study of patients infected during the inaugural (2003) WNV season in Alberta, Canada.

Fifty patients who were WNV IgM positive by 2 commercial IgM kits (West Nile virus capture EIAs, Focus Technologies, Cypress, CA, USA, and Panbio, Windsor, Queensland, Australia) during the fall of 2003 were contacted. Sera were recollected and tested for IgM and IgG antibodies to WNV with current kits from these 2 companies. Sera were also tested for hemagglutination-inhibiting (HI) antibodies to WNV (2).

Of 39 serum samples from 38 patients, 28 were positive, 5 were indeterminate, and 6 were negative with the Focus IgM kit. Twenty-one were positive, 3 were indeterminate, and 14 were negative with the Panbio IgM kit. All had WNV IgG antibodies detected by Focus and Panbio IgG kits. We detected HI antibodies to WNV in all patients, and titers in 12 were  $\geq 320$ . The time course for IgM index values for the Focus IgM kit used in 2003 and 2004 is shown in the Figure.

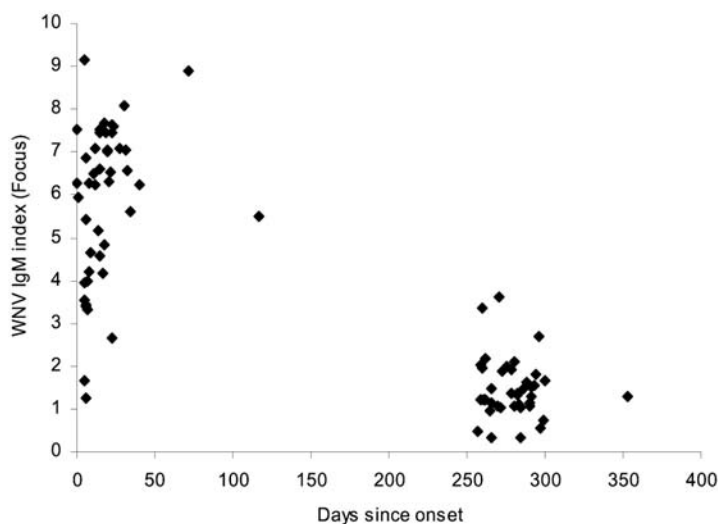


Figure. West Nile virus (WNV) immunoglobulin (IgM) index values in serum specimens from 38 WNV case-patients detected in the fall of 2003. The assay was performed by using the Focus Technologies kit, as per the manufacturer's instructions. An index  $>1.1$  indicates a positive result and an index  $<0.9$  indicates a negative result.

These data show that when tests are conducted with newly available kits, as with the CDC in-house test, IgM antibody to WNV persists for  $\geq 8$  months in most patients. A single high HI titer is not helpful in identifying recent infection. In addition, the IgM test cannot differentiate between recent and past infections. Interpreting a positive IgM result in WNV-endemic areas will be complex because a positive WNV IgM result could indicate a current acute infection or a previous WNV infection even in a person with a different acute illness.

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## Hepatitis A, Italy

**To the Editor:** Hepatitis A virus (HAV) infection rates are very low in industrialized countries. A noticeable fall in the prevalence of HAV antibodies (anti-HAV) has been reported in southern European and Mediterranean countries such as Spain (1) and Greece (2), reflecting improvements in hygiene standards in the last decades.

An HAV prevalence of 66.3% in 1981 (3) and 29.4% in 1990 (4) was shown in studies conducted in military recruits from all Italian regions. In both studies, subjects from southern regions had a higher HAV prevalence than those from north-central regions. In 2003, we conducted a study of recruits to show changes in HAV infection prevalence in younger Italian generations.

Military service was compulsory in Italy at that time; all men 18–26 years of age were included. From September to December 2003, 323 recruits 18–26 years of age (mean age 20 years), representing all Italian regions, who had been accepted for Air Force military service were tested