

A case of dengue type 3 virus infection imported from Africa to Italy, October 2009

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Citation style for this article:

Citation style for this article: Nisii C, Carletti F, Castilletti C, Bordi L, Meschi S, Selleri M, Chiappini R, Travaglini D, Antonini M, Castorina S, Lauria FN, Narciso P, Gentile M, Martini L, Di Perri G, Audagnotto S, Biselli R, Lastilla M, Di Caro A, Capobianchi M, Ippolito G. A case of dengue type 3 virus infection imported from Africa to Italy, October 2009. *Euro Surveill.* 2010;15(7):pii=19487. Available online: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19487>

This article has been published on 18 February 2010

In October 2009, a traveller returning from Africa to Italy was hospitalised with symptoms suggestive of a haemorrhagic fever of unknown origin. The patient was immediately placed in a special biocontainment unit until laboratory investigations confirmed the infection to be caused by a dengue serotype 3 virus. This case reasserts the importance of returning travellers as sentinels of unknown outbreaks occurring in other countries, and highlights how the initial symptoms of dengue fever resemble those of other haemorrhagic fevers, hence the importance of prompt isolation of patients until a final diagnosis is reached.

Case description

In October 2009, a Senegalese man in his forties, who had been living in Italy for 20 years, presented to Hospital A in the northern Italian city of Turin three days after returning (via Madrid) from a four-month visit to his home village in Senegal. During his stay, he had never left the village and had not visited any healthcare centres. The initial symptoms of disease, a persistent fever (>38 °C) accompanied by an unremitting headache, started during the flight from Madrid to Turin worsened over the previous two days despite treatment with paracetamol.

On the day after hospitalisation, when laboratory test results showed a platelet count of 5,000 cells/mm³ as well as evidence of altered liver function (aspartate aminotransferases (AST): 3,539 U/L; alanine aminotransferases (ALT): 815 U/L; lactate dehydrogenase (LDH) 3,609 U/L; gamma-glutamyl transferases (gamma-GT): 112 U/L), the patient was first transferred to an infectious diseases hospital in Turin (Hospital B), and two days later to the National Institute for Infectious Diseases in Rome (Hospital C), which is the Italian national reference centre for emerging infections and bioterrorism, on suspicion of a viral haemorrhagic fever. The patient was admitted to a special biocontainment unit during the same night. Over the following

days, the patient's clinical condition improved and he was discharged nine days after admission.

Laboratory investigations

After the patient's admission, series of tests were run in parallel on samples taken at Hospital C and in Turin, before the transfer to Rome. The tests included in the differential diagnosis ruled out malaria (which had already been excluded in Turin), as well as infection with the major agents of viral hepatitis, herpes simplex virus and viral haemorrhagic fever viruses. Serological investigation using an in-house immunofluorescence assay (slides prepared with a mix of uninfected and dengue type 2-infected Vero cells) revealed a high IgG titre against dengue virus soon after the onset of symptoms (in a sample taken on 10 October), with the presence of IgM antibodies at a low titre, and RT-PCR followed by sequencing of the NS5 region confirmed the infectious agent to be a serotype 3 dengue virus. In addition, a 224 bp fragment of the E gene was amplified and sequenced. The phylogenetic analysis of this fragment is shown in the Figure. Our patient was infected with a strain belonging to dengue virus serotype 3, genotype III closely related to other strains found in Africa, but never reported from Senegal before the case described here [1].

The tests carried out the day before the patient left the hospital showed that his IgG titre was still increasing. Overall, the immune response was consistent with a re-infection with a dengue serotype 3, genotype III virus which can lead to a severe form of the disease.

Case management

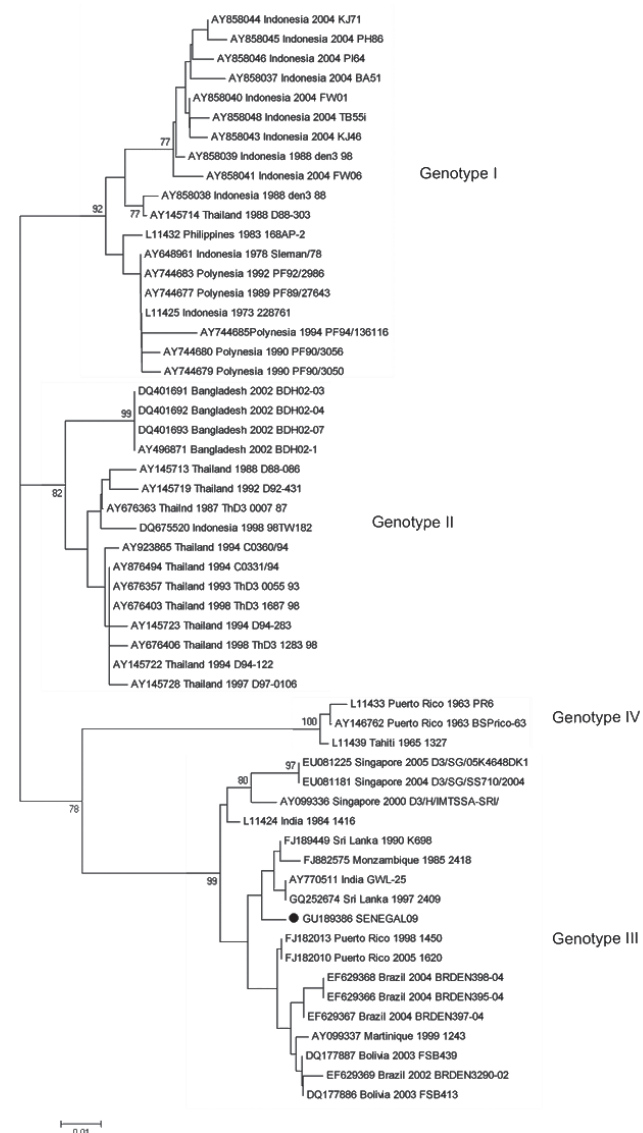
At the time the patient reported to the first hospital in Turin, there was no information on any outbreaks of dengue virus taking place in Senegal [1]. Therefore, the fact that the patient developed symptoms suggestive of a haemorrhagic disease led the clinicians to suspect also other, more dangerous viral infections, namely Lassa virus or Crimean-Congo haemorrhagic fever

virus, both categorised as biosafety level 4 (BSL4) agents [2-3]. As these infections could not be ruled out on clinical and epidemiological criteria, it was decided to transfer the patient to a high-security isolation facility.

When contacted by Hospital B in Turin, the National Institute for Infectious Diseases immediately requested the shipping of clinical samples, but because of logistical difficulties the transport could not be organised.

FIGURE

Phylogenetic relationship of the strain isolated in October 2009 in Rome (identified by the black dot) with other dengue serotype 3 virus strains described elsewhere [10]



Sequence identification is as follows: strain name, country and year of isolation. The scale bar indicates nucleotide substitutions per site. Multiple alignment of our sequence and other dengue serotype 3 virus sequences available in GenBank was generated with ClustalW1.7 software included in the Bioedit package. The phylogenetic tree was constructed by using nucleotide alignment, the Kimura-2-parameters algorithm, and the neighbour-joining method implemented in the MEGA 4.1 software. The robustness of branching patterns was tested by 1,000 bootstrap pseudoreplications.

It was then decided by the Italian Ministry of Health, in consultation with the institute, to make use of the procedure already in place in Italy for the transport of highly infectious patients under high isolation conditions, which included the use of military aircraft and equipment of the Italian Air Force and Hospital C (stretcher isolators and a high containment ambulance). On arrival, the patient was immediately transferred to the biocontainment unit and was attended to by specially trained, dedicated staff employing all necessary biosafety precautions.

Conclusions

We have described a case of dengue virus infection imported into Italy from Africa, which to date has been a rare occurrence as most cases seen in European travel clinics have been imported either from Asia or the Americas. Dengue virus has been known to circulate in parts of Africa for decades, and Senegal in particular has experienced several outbreaks, mainly of dengue serotype 2 virus [1]. Following our report [4], which was the first for a Dengue serotype 3 virus in Senegal, the country's health authorities were alerted to a possible epidemic and since then, over 50 cases have been registered in the country [5-6], confirming the importance of returning travellers as sentinels of as yet unreported outbreaks occurring abroad [7].

From a clinical point of view, the patient did not meet the World Health Organization's (WHO) criteria for severe dengue fever [8], but presented a set of warning signs indicative of haemorrhagic fever such as liver enlargement (>2 cm), altered liver enzymes (20-fold) and a rapid decrease in platelet count. Therefore, and because the information available at the time did not allow excluding the involvement of a BSL4 agent, the decision to treat the case as a potential viral haemorrhagic fever was made early in the management process. The recent report of a case of Marburg haemorrhagic fever imported from Uganda into the United States [9] that was diagnosed retrospectively in a serum sample archived six months earlier after it had tested negative for agents of viral haemorrhagic fever, should teach us that in similar cases, patients should immediately be placed in an adequate containment facility until a final diagnosis is reached. Our recommendation is to perform all diagnostic procedures safely at the appropriate biosafety level, and as a general rule any activities involving patient samples at a level lower than BSL3 should be kept to a minimum. It remains a problem that the transport of samples is still an unsolved issue in Italy as well as in Europe and worldwide. The timely shipping of biological samples in advance of the patient's arrival would have shortened the time to reach a diagnosis and avoided the extra costs of unnecessary biosafety measures.

References

1. World Health Organization. Dengue in Africa: emergence of DENV-3, Côte d'Ivoire, 2008. *Wkly Epidemiol Rec.* 2009;84(11-12):85-8. Available from: http://www.who.int/wer/2009/wer8411_12.pdf
2. Laboratory Biosafety Manual. Second edition (revised). Geneva: World Health Organisation; 2003. WHO/CDS/CSR/LYO/2003.4. Available from: <http://www.who.int/csr/resources/publications/biosafety/Labbiosafety.pdf>
3. JY Richmond, RW McKinney, editors. Biosafety in Microbiological and Biomedical Laboratories. Fourth edition. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention and National Institutes of Health; 1999. Available from: <http://www.cdc.gov/od/ohs/pdffiles/4th%20BMBL.pdf>
4. Hemorrhagic fever – Italy ex Senegal (02): dengue. In: ProMED-mail [online]. Boston US: International Society for Infectious Diseases; 16 October 2009. Archive no. 20091016.3559. Available from: http://www.promedmail.org/pls/apex/f?p=2400:1001::NO::F2400_P1001_BACK_PAGE,F2400_P1001_PUB_MAIL_ID:1000%2C79642
5. Dengue/DHF Update 2009 (47). In: ProMED-mail [online]. Boston US: International Society for Infectious Diseases; 15 November 2009. Archive no. 20091115.3944. Available from: http://www.promedmail.org/pls/apex/f?p=2400:1001::NO::F2400_P1001_BACK_PAGE,F2400_P1001_PUB_MAIL_ID:1000%2C80100
6. Dengue/DHF Update 2009 (48). In: ProMED-mail [online]. Boston US: International Society for Infectious Diseases; 23 November 2009. Archive no. 20091123.4016. Available from: http://www.promedmail.org/pls/apex/f?p=2400:1001::NO::F2400_P1001_BACK_PAGE,F2400_P1001_PUB_MAIL_ID:1000%2C80198
7. Schwartz E, Weld LH, Wilder-Smith A, von Sonnenburg F, Keystones JS, Kain KC, et al. Seasonality, annual trends, and characteristics of dengue among ill returned travellers, 1997-2006. *Emerg Infect Dis.* 2008;14(7):1081-8.
8. Dengue: Guidelines for diagnosis, treatment, prevention and control. New edition. Geneva: World Health Organization, 2009. ISBN 978 92 4 154787 1. WHO/HTM/NTD/DEN/2009.1. Available from: http://whqlibdoc.who.int/publications/2009/9789241547871_eng.pdf
9. Marburg hemorrhagic fever – USA ex Uganda 2008. In: ProMED-mail [online]. Boston US: International Society for Infectious Diseases; 31 January 2009. Archive no. 20090131.0423. Available from: http://www.promedmail.org/pls/apex/f?p=2400:1001::NO::F2400_P1001_BACK_PAGE,F2400_P1001_PUB_MAIL_ID:1000%2C75888
10. Weaver SC, Vasilakis N. Molecular evolution of Dengue viruses: contributions of phylogenetics to understanding the history and epidemiology of the preeminent arboviral disease. *Infect Genet Evol.* 2009;9(4):523-40.