

FIRST HUMAN CASE OF USUTU VIRUS NEUROINVASIVE INFECTION, ITALY, AUGUST-SEPTEMBER 2009

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We report the first worldwide case of Usutu virus (USUV) neuroinvasive infection in a patient with diffuse large B cell lymphoma who presented with fever and neurological symptoms and was diagnosed with meningoencephalitis. The cerebrospinal fluid was positive for USUV, and USUV was also demonstrated in serum and plasma samples by RT-PCR and sequencing. Partial sequences of the premembrane and NS5 regions of the viral genome were similar to the USUV Vienna and Budapest isolates.

Introduction

Usutu virus (USUV) is an arthropod-borne virus of the family *Flaviviridae*, genus *Flavivirus*. It is included in the Japanese encephalitis virus (JEV) group [1] being closely related to human pathogens such as JEV and West Nile virus (WNV). In the last decade, USUV was detected in a variety of central European birds with encephalitis, myocardial degeneration, and necrosis in liver and spleen [2-5]. As far as we know, the virus had never been associated with severe or fatal disease in humans [6]; it was isolated once in the Central African Republic in a man with fever and rash [7]. Here we report evidence of a neuroinvasive infection clinically related to USUV in Italy.

Case report

In May 2009, a woman in her 60s from Emilia Romagna region, Italy, underwent hemicolectomy because of a diffuse large B cell lymphoma. Six courses of chemotherapy were administered (including rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone), with last administration on 21 August 2009. Some days later, there was a reactivation of genital herpes treated with valacyclovir. On 1 September, a fever of 39.5°C with resting tremor appeared and antibiotic (moxifloxacin and amoxicillin clavulanate) therapy started however the temperature persisted. On 5 September, the patient was admitted to hospital for hyperpyrexia resistant to antipyretic and intravenous antibiotic treatment (meropenem and teicoplanin). Once admitted, the patient received blood transfusion because of a critical anaemia.

Examination of blood, urine and stool cultures and virological assessment for herpes virus simplex (HSV1/2) and cytomegalovirus

(CMV) antigen were negative. A total body computerised tomography was performed without evidence of lymphoma. Suspicion of meningoencephalitis was addressed by neurological examination which showed distal resting tremor, positivity to the Romberg test, dysmetria and weakness at four limbs without cranial nerve affection. Magnetic resonance imaging (MRI) of the brain showed a signal alteration of the substantia nigra of the parietal and frontal subcortical areas that did not change after injection of contrast medium. On 11 September, the cerebrospinal fluid (CSF) was therefore collected and examined. The CSF was limpid without any alteration detected in the clinical-chemical analysis, activated lymphocytes were evident in the sediment. As further analysis of the same CSF specimen revealed the presence of flaviviruses (see below), steroid treatment was started. This therapy resolved the fever but did not lead to any improvement of the neurological symptoms. The electroencephalogram still registered diffuse slow theta waves and slow spike prevalent in left frontal parietal areas. The neurological functions, mainly the resting tremor, improved following the administration of levodopa and carbidopa.

Virological analysis

When tested for the presence of viral agents, the CSF collected on 11 September was negative in molecular tests for CMV, HSV1/2, Epstein-Barr virus, adenoviruses, parvovirus B19, polyomavirus JC and BK, enteroviruses, mumps virus and WNV and positive to a heminested RT-PCR specific for the NS5 region of the *Flavivirus* genus [8]. The amplicon was directly sequenced and analysed by BLAST (<http://www.ncbi.nlm.nih.gov/blast>), revealing a 98% identity with both the USUV Budapest (gbIEF206350.1) and Vienna (gbAY453411.1) isolate.

To confirm the identification of the species Usutu virus, we performed two USUV-specific RT-PCRs targeting the NS5 [2] and premembrane (preM) regions (primer sequences available on request) of the USUV genome on two plasma specimens collected on 8 and 11 September 2009 and one serum specimen collected on 14 September. The amplified products were sequenced (583 bp of NS5 and 602 bp of preM) and aligned with the corresponding sequences deposited in Genbank (gbAY453411.1;

gbIEF206350.1) using ClustalW. The alignment of the preM gene shared 99% nucleotide identity with the USUV Budapest and Vienna sequences, whereas the NS5 gene sequences shared 100% nucleotide identity with USUV Vienna and 99% with USUV Budapest.

Further specimens of serum (26 May and 13 October) and plasma (19 October) before and after the acute phase of meningoencephalitis were analysed to demonstrate the absence of the virus. The two USUV-specific RT-PCRs performed on these three samples did not detect any USUV RNA. These samples were also analysed for WNV because a WNV outbreak was ongoing in the area at the time [9], and were negative.

Discussion

To our best knowledge this is the first human disease with neurological involvement caused by USUV. The detection of USUV only in those samples collected during the acute phase of clinical manifestation is clear evidence that the virus caused the meningoencephalitis in the patient. Its capability of causing neurological lesions and death has already been reported in birds of central Europe [10]. The presence of USUV in Emilia Romagna has also been reported [4] and, in the past few months, the virus was isolated from black birds found dead in Northern Italy [G. Savini, personal communication 22 October 2009]. A surveillance programme in sentinel chicken flocks to monitor the possible appearance and/or circulation of WNV and other flaviviruses has been in place for several years. In the clinical case reported here, the immunosuppressed status of the patient due to both the underlying disease and the treatment, particularly with rituximab, may have played an important role in USUV infection and in its pathogenicity. It is known that rituximab can reactivate hepatitis B virus in patients with lethal fulminant hepatitis.

However, a possible unusual neuroinvasiveness and neurovirulence of this particular USUV strain cannot be excluded. The fact that neurological symptoms occurred prior to hospital admission excludes the transfusion as a possible source of infection. Conversely, since USUV as well as competent viral vectors are circulating in the patient's area of residence [4], it is likely that the infection was transmitted to the patient through mosquito bites.

References

1. Heinz FX, Collett MS, Purcell RH, Gould EA, Howard CR, Houghton RJ, et al. Family Flaviviridae. Virus Taxonomy. Seventh Report on International Committee on Taxonomy of Viruses. van Regenmortel MHC, Fauquet CM, Bishop DHL, Carstens EB, Estes MK, Lemon SM, et al, editors. San Diego, San Francisco, New York, Boston, London, Sydney, Tokyo: Academic Press; 2000. p. 859-78.
2. Bakonyi T, Erdélyi K, Ursu K, Ferenczi E, Csörgö T, Lussy H, et al. Emergence of Usutu virus in Hungary. *J Clin Microbiol.* 2007;45(12):3870-4.
3. Chvala S, Kołodziejek J, Nowotny N, Weissenböck H. Pathology and viral distribution in fatal Usutu virus infections of birds from the 2001 and 2002 outbreaks in Austria. *J Comp Pathol.* 2004;131(2-3):176-85.
4. Lelli R, Savini G, Teodori L, Filippini G, Di Gennaro A, Leone A, et al. Serological evidence of USUTU virus occurrence in north-eastern Italy. *Zoonoses Public Health.* 2008; 55(7): 361-7.
5. Manarolla G, Bakonyi T, Gallazzi D, Crosta L, Weissenböck H, Dorrestein GM, et al. Usutu virus in wild birds in northern Italy. *Vet Microbiol.* 2009; Aug 8.
6. Weissenböck H, Hubálek Z, Bakonyi T, Nowotny N. Zoonotic mosquito-borne flaviviruses: Worldwide presence of agents with proven pathogenicity and potential candidates of future emerging diseases. *Vet. Microbiol.* 2009; Aug 26.
7. Adam F, Diguette J-P. Virus d'Afrique [base de données].[Internet]. Dakar: Institut Pasteur de Dakar. Centre collaborateur OMS de référence et de recherche pour les arbovirus et les virus de fièvres hémorragiques (CRORA). Available from: <http://www.pasteur.fr/recherche/banques/CRORA>
8. Scaramozzino N, Crance JM, Jouan A, Debriél DA, Stöll F, Garin D. Comparison of Flavivirus universal primer pairs and development of a rapid, highly sensitive heminested reverse transcription-PCR assay for detection of flaviviruses targeted to a conserved region of the NS5 gene sequences. *J. Clin. Microbiol.* 2001;39(5):1922-7.
9. Rizzo C, Vescio F, Declich S, Finarelli AC, Macini P, Mattivi A, et al. West Nile virus transmission with human cases in Italy, August - September 2009. *Euro Surveill.* 2009;14(40):pii=19353. Available from: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19353>
10. Weissenböck H, Kołodziejek J, Url A, Lussy H, Rebel-Bauder B, Nowotny N. Emergence of Usutu virus, an African mosquito-borne flavivirus of the Japanese encephalitis virus group, central Europe. *Emerg Infect Dis.* 2002;8(7):652-6.