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 (moxifloxacine and amoxicilline with valacyclovir). 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 (gb|AY453411.1).

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, parovirus 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 (gb|EF206350.1) and Vienna (gb|AY453411.1) 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 (gb|AY453411.1).
gb|EF206350.1) 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**