The First Case of Usutu Virus Neuroinvasive Disease in Austria, 2021

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Usutu virus (USUV) is a mosquito-borne flavivirus closely related to West Nile virus (WNV) that is endemic in many European countries. We report the first case of USUV neuroinvasive disease in Austria and discuss challenges in differentiating USUV from WNV infections in areas where both viruses are endemic.

Keywords. arbovirus; neuroinvasive disease; Usutu virus; viral meningitis.

Usutu virus (USUV) is as mosquito-borne flavivirus that was introduced to Europe approximately 20 years ago and is now endemic in many European countries [1], including in Austria since 2001 [2–7]. Although USUV infection usually causes asymptomatic infection in humans, neuroinvasive disease has been described in single patients [8–10]. USUV is closely related to West Nile virus (WNV), another mosquito-borne flavivirus endemic to southern Europe, which renders diagnosis difficult due to serological cross-reactivity. Both viruses are members of the Japanese encephalitis serogroup of flaviviruses, and both have similar zoonotic hosts, circulating in birds with *Culex* mosquitoes as the major vectors. USUV infection of some species of birds, especially common blackbirds (*Turdus merula*), has been associated with high mortality and mass die-offs [11]. Here we report the first Usutu virus neuroinvasive disease (UND) case in Austria and discuss virological diagnostic challenges.

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CASE REPORT

An 81-year-old man living in Vienna, Austria, was referred to the hospital in early September 2021 after a witnessed sudden fall without loss of consciousness, according to his daughter who observed the incident. Seven days prior to the incident he had experienced fever and malaise and had been diagnosed with suspected otitis media, and antibiotic treatment (amoxicillin-clavulanic acid) had been started. Upon admission, the patient was febrile (temperature 39.9°C). He displayed confusion and could not respond to questions properly. Notable medical history included hypertension, left-sided hemifacial spasms, and bilateral deafness secondary to meningitis in childhood. Daily medications included antihypertensive treatment with an angiotensin-converting enzyme inhibitor and a β-blocker. The patient's relatives reported no recent travel history, and confirmed that he was otherwise healthy and led an active lifestyle including outdoor sports. The patient had never experienced any flavivirus infections and had never been vaccinated against any flavivirus, including tick-borne encephalitis virus, Japanese encephalitis, or yellow fever. Physical examination revealed no focal neurologic deficits. Laboratory workup demonstrated leukocytosis (17.0 G/L) with neutrophilia (85%) as well as elevated C-reactive protein, interleukin 6, and creatine kinase. Urine tests (pneumococcus and *Legionella* antigen, urine culture), blood cultures, and severe acute respiratory syndrome coronavirus 2 reverse-transcription polymerase chain reaction (RT-PCR) were negative. A chest radiograph revealed a right lower lobe pneumonia, leading to initiation of antibiotic medication. Intracranial pathologies, including ischemic stroke, intracranial hemorrhage, or potential traumatic fractures, were excluded by head computed tomography (CT), head/neck CT angiogram, and magnetic resonance imaging (MRI) of the brain. A lumbar puncture performed at time of hospital admission showed an increased cerebrospinal fluid (CSF) cell count with lymphocytic pleocytosis (74 leukocytes/µL) and protein levels (528 mg/L) indicative of a disturbed blood-brain barrier, while intrathecal antibody synthesis was excluded. Glucose level was normal and oligoclonal bands were positive in CSF and serum. Autoimmune encephalitis was ruled out by lack of evidence for antineuronal antibodies in the CSF and serum. These samples were tested further for the presence of pathogens (below).

Despite antibiotic treatment, the patient's status rapidly deteriorated and intubation in the intensive care unit was required due to hypercapnia. Positive cultures for Enterobacteriaceae as well as *Klebsiella pneumoniae* in tracheal secretions on day 7 after hospital admission were linked to hospital-associated infection and led to a change of treatment to meropenem, resulting in clinical improvement. As the patient required little respiratory support, quick weaning was pursued and sedation

was reduced on day 4 after hospitalization. However, the patient developed intermittent clonic jerks of his right upper extremity. Electroencephalographic (EEG) monitoring showed one seizure beginning in the left frontotemporal area and generalized spreading with rhythmic polyspike/spike-wave activity. Concomitantly, chewing movements and gaze deviation to the right were recorded. Antiepileptic therapy with lorazepam, levetiracetam, and valproic acid was initiated as repeated EEGs revealed persistent ictal-interictal continuum with continuous generalized delta activity (1.5 Hz/s) consistent with severe encephalopathy. Upon switch to lacosamide and perampanel, EEG improved and clinical seizures remitted. Repeated neurologic examinations showed no meningeal signs, small isocoric pupils, no vestibulo-ocular reflex, normal reflexes and relaxed muscle tonus in all extremities, negative Babinski sign on both sides, and no reaction to painful stimuli.

While the first cranial MRI scan yielded inconspicuous results, hyperintensities in the left hippocampal region on T-weighted images were noted 26 days after admission that were consistent with limbic encephalitis (Figure 1). Subsequent MRI showed regression of these lesions, though large confluent white matter lesions in both hemispheres (not shown) and cerebral atrophy were seen (Figure 1). Repeated lumbar punctures revealed intrathecal immunoglobulin G (IgG) synthesis 34 days posthospitalization while leukocyte count continuously decreased. A CSF analysis on day 79 still showed intrathecal antibody production with mild pleocytosis (6 cells/μL) (Supplementary Table). At the time of article submission (nearly 7 months after admission), the tetraparetic patient continues to require constant monitoring and hospital care. He is awake, can be stimulated by emotional triggers, and does not further require ventilation support.

VIROLOGICAL RESULTS

No pathogens were detected in CSF by a qualitative multiplex PCR (BioFire FilmArray Meningitis/Encephalitis Panel, bioMérieux, France) reported to detect 14 pathogens commonly associated with meningitis/encephalitis: human cytomegalovirus, enteroviruses, herpes simplex virus 1 and 2, human herpesvirus 6, human parechovirus, varicella zoster virus, *Escherichia coli* K1, *Haemophilus influenzae*, *Listeria monocytogenes*, *Neisseria meningitidis*, *Streptococcus agalactiae*, *Streptococcus pneumoniae*, and *Cryptococcus neoformans/gattii*. Serum and CSF samples 20 days after hospital admission were positive for WNV immunoglobulin M (IgM) and IgG antibodies by enzyme-linked immunosorbent assay (ELISA) (Table 1). However, WNV-specific RT-PCR was negative throughout the course of disease in serum, whole blood, CSF, and urine. As it is known that antibodies against WNV and USUV are cross-reactive in ELISA tests and USUV is endemic in Austria, CSF and sera were tested via USUV-specific RT-PCR [11]. USUV
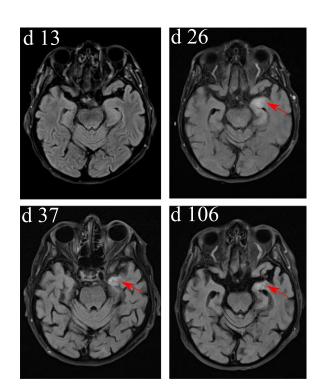

Figure 1. Axial fluid-attenuated inversion recovery magnetic resonance images (T2-weighted) of a patient with Usutu virus neuroinvasive disease taken after hospital admission. Day of hospital stay is indicated on the top left corner of each panel. No clear abnormalities were seen on day 13 after hospital admission (top left). Hyperintense alterations suggestive of limbic encephalitis were noted predominantly in the left hippocampal/parahippocampal area (arrow, top right image, day 26). These alterations were slowly regressive on subsequent days postadmission (arrows in bottom left and bottom right images).

RNA was detected in the first CSF sample collected at hospital admission (Table 1). A partial sequence (524 nt) was obtained [12] and determined to be 100% identical to sequences obtained from humans, birds, and mosquitoes in the Czech Republic, Hungary, and Austria by nucleotide BLAST search, all in the "Europe 2" lineage (GenBank Accession OM142204). Virus neutralization tests (NTs) were determined from all serum samples collected during hospital stay against WNV or USUV [4, 5]. While the WNV NT was negative (<1:20) for all samples, neutralizing antibodies against USUV were detected 4 days posthospitalization (1:20) and increased to 1:40 on days 20 and 27 posthospitalization (Table 1). Analysis of serum by ELISA showed no IgM or IgG reactive against tick-borne encephalitis virus. The presence of USUV RNA in CSF, the development of neutralizing antibodies against USUV, and the lack of neutralization against WNV indicated an acute USUV neuroinvasive infection.

DISCUSSION

USUV emerged in Austria in 2001, marked by deaths in several bird species [2]. The virus remains endemic in Austria with

Table 1. Virological and Serological Results From an Usutu Virus Neuroinvasive Disease Patient in Austria, 2021

Day Posthospitalization	WNV Serum PCR[a]	WNV Serum IgM[b]	WNV Serum IgG[b]	WNV Serum NT[c]	WNV CSF PCR[a]	WNV CSF IgM[b]	WNV CSF IgG[b]	WNV Urine PCR	USUV Serum PCR[a]	USUV Serum NT[c]	USUV CSF PCR[a]	USUV Urine PCR[a]	TBEV Serum IgG	TBEV Serum IgM
0	neg	neg	neg	<20	neg	ND	ND	NA	neg	<20	pos	NA	neg	neg
4	neg	pos	pos	<20	NA	NA	NA	NA	neg	20	NA	NA	neg	neg
20	neg	pos	pos	<20	neg	pos	pos	NA	neg	40	neg	NA	neg	neg
22	neg[d]	ND	ND	ND	NA	NA	NA	neg	neg[d]	ND	NA	neg	NA	NA
27	neg	pos	pos	<20	NA	NA	NA	neg	neg	40	NA	neg	NA	NA

Abbreviations: CSF, cerebrospinal fluid; IgG, immunoglobulin G; IgM, immunoglobulin M; NA, sample not available; ND, not determined, neg, negative/not detected; NT, neutralization test; PCR, polymerase chain reaction; pos, positive/detected; TBEV, tick-borne encephalitis virus; USUV, Usutu virus; WNV, West Nile virus.

[a]Qualitative reverse-transcription PCR for WNV and USUV were performed as previously described [5, 11].

[b]WNV-IgG enzyme-linked immunosorbent assay (ELISA) was performed with a commercial kit (EUROIMMUN AG, Luebeck, Germany) and WNV-IgM ELISA was performed with an in-house assay as previously described [4].

[c]Neutralization tests were performed as previously described [4, 6].

[d]PCR was performed on nucleic acids extracted from whole blood in ethylenediaminetetraacetic acid.

annual variation in incidence in the human, mosquito, and bird populations [6, 7]. Pathological and virological examinations of dead birds (including blackbirds, *Turdus merula*) and experimentally infected mice have supported the neuroinvasive potential of USUV [12, 13]. The first human cases of UND were reported in Italy in 2009, and since then more UND cases in Europe have been detected in immunocompromised as well as in immunocompetent patients, potentially due to increased awareness of the disease, increased geographic spread of the virus, and/or annual variation in enzootic transmission activity [8–10, 14–19]. Retrospective studies conducted in Italy (2008–2011) found higher infection ratios for USUV than for WNV in patients with undiagnosed neurological impairments [10, 15].

The few clinically described patients with USUV-associated meningitis all had favorable outcomes and fully recovered [16]. However, patients with neuroinvasive USUV infection leading to meningoencephalitis all experienced lasting residual effects: A previously healthy 29-year-old woman reported persistent headaches and showed deficits in declarative memory and speech fluency [10, 15], and a woman in her forties had a similar outcome to our patient [10, 15]. The most common initial clinical features were those typical for meningoencephalitis including headache, high fever, nuchal rigidity, and impaired consciousness, often followed by impaired motor functions manifesting as tremor, dysmetria, ataxia, or dysarthria.

Clinically, UND cannot be differentiated from WNV-associated neuroinvasive disease; therefore, documented cases are needed for a more detailed characterization. USUV RNA has been detected in donor blood samples in Austria since 2016, all of whom were asymptomatic excluding a single report of a rash [5, 6]. In the case reported here, we note that without the identification of USUV RNA in the CSF, the patient would have met the criteria to be classified as a West Nile neuroinvasive disease case (ie, detection of WNV-reactive IgM in the CSF) [20]. We have noted that neutralization titers were useful in differential diagnosis in this case and in others [6]. Physicians and virologists in endemic regions should consider UND a possible infectious cause for meningitis or encephalitis of unknown origin, especially during late summer months. Of primary importance is the implementation of molecular diagnostics, particularly for screening donor blood, to ensure both USUV and WNV are capable of being detected.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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