Clinical and laboratory characteristics of neuroinvasive viral zoonoses detected in continental Croatian regions, 2017-2018

Tatjana Vilibic-Cavlek¹, Maja Bogdanic¹, Vladimir Savic¹, Irena Tabain¹, Ljiljana Peric¹, Dario Sabadi², Tanja Potocnik-Hunjadi¹, Bozana Miklausic¹, Sanja Zember¹, Marija Santini², Gordana Kolaric-Sviben², Elizabeta Dvorski¹, Tamara Butigan², Lorena Jemersic¹, Jelena Prpic¹, Branko Kolaric¹, Marina Balicevic¹, Vladimir Stevanovic¹, Ljubo Barbic¹, Andrea Babic-Erceg¹, Eddy Listes², Josip Madic¹, Ana Klobucar², Giovanni Savini^{2,3}

Arbovirus study group; ¹Collaborators on the project Croatian Science Foundation: IP-2016-06-7456: "Prevalence and molecular epidemiology of emerging neuroinvasive arboviral infections in Croatia" (CRONEUROARBO); ²Collaborators of the Reference Center for Diagnosis and Surveillance of Viral Zoonoses Croatian Ministry of Health; Croatia; ³OIE Reference Centre for West Nile Disease, Istituto Zooprofilattico Sperimentale "G. Caporale", Teramo, Italy

Among neuroinvasive zoonotic viruses, tick-borne encephalitis virus (TBEV) and West Nile virus (WNV) are the most widely distributed. Usutu virus (USUV) is detected in many bird species in Europe, however, human neuroinvasive infections are rarely reported. Althouht many Toscana virus (TOSV) infections are asymptomatic or presented with influenza-like symptoms, the virus displays a strong neurotropism. Outbreaks of TOSV meningitis were reported in several European countries bordering the Mediterranean Sea. Meningitis occurs occasionally during Tahyna virus (TAHV) infection. Lymphocytic choriomeningitis virus (LCMV) is a neglected virus which may cause aseptic meningitis, especially in immunocompromised persons.

Purpose:

The aim of this study was to analyze clinical and laboratory characteristics of neuroinvasive viral zoonoses detected in Croatia during 2017-2018.

Methods and Materials:

From April 2017 to June 2018, a total of 120 patients with neuroinvasive disease from continental Croatian regions (fig.1) were tested for the presence of neuroinvasive zoonotic viruses: TBEV, WNV, USUV, TOSV, TAHV and LCMV. Cerebrospinal fluid (CSF) and urine samples were tested for the presence of viral RNA using a real-time RT-PCR and/or nested RT-PCR. Serological tests of serum and CSF samples (IgM/IgG antibodies, IgG avidity) were performed using enzyme-linked immunosorbent assay (TBEV, WNV, USUV), indirect immunofluorescence assay (IFA; TOSV, LCMV) and virus neutralization test (WNV). Commercial ELISA and IFA (Euroimmun, Lübeck, Germany) were used for detection of TBEV, WNV, USUV and TOSV antibodies. LCMV Armstrong strain was used as antigen for *in-house* IFA.

Results:

Etiology was confirmed in 28/23.3% patients. TBEV was documented in 20/16.7% and WNV in 8/6.6% patients by detection of IgM and IgG antibodies of low avidity and/or detection of viral RNA in CSF and urine samples. Majority of patients with TBEV infection were males (15/75.0%). Although infections were detected in all age groups, 15/75.0% patients were less than 60 years of age. The main clinical symptoms were headache (18/90.0%), weakness (18/90.0%), nausea (12/60.0%) and vomiting (8/40.0%). Fever >39°C was noted in 16/80.0% patients. CSF leukocyte count ranged from 41-3520/mm³ with mononuclear cell predominance in 15/75.0% patients. All but one patient fully recovered. WNV infection was reported in 5/62.5% males and 3/37.5% females. All but one patient (7/87.5%) were older than 60 years. Majority of patients reported underlying diseases: hypertension (3/37.5%), cerebrovascular disease (3/37.5%) and diabetes (1/12.5%). The main clinical symptoms were headache (5/62.5%) and weakness (5/62.5%), while fever >39°C was noted in 4/50.0% patients. CSF leukocyte count ranged from 56-1096/mm³ with mononuclear cell predominance in 4/50.0% patients. One patient died. Acute USUV, TOSV, TAHV and LCMV infections were not detected during the tested period.







Table 2. Laboratory characteristics of patients with neuroinvasive infection

Parameter	TICK-BORNE ENCEPHALITIS	WEST NILE INFECTION
Leukocyte count (range)	41 - 3520/mm ³	56 - 1096/mm³
Protein level (range)	0.717 - 1.107 g/L	0.617 - 1.049 g/L
Mononuclear cell predominance	15 (75.0%)	4 (50.0%)

Conclusions:

TBEV infections were more common in patients less than 60 years of age,

Table 1. Demographic and clinical characteristics of patients with neuroinvasive infection

Characteristic	TICK-BORNE ENCEPHALITIS	WEST NILE INFECTION
Gender Male Female	15 (75.0%) 6 (25.0%)	5 (62.5%) 3 (37.5%)
Age group <20 years 20-39 years 40-59 years 60+ years	1 (5.0%) 4 (20.0%) 10 (50.0%) 5 (25.0%)	0 (0%) 1 (12.5%) 0 (0%) 7 (87.5%)
Underlying diseases Hypertension Cerebrovascular disease Diabetes	4 (20.0%) 2 (10.0%) 0 (0%)	3 (37.5%) 3 (37.5%) 1 (12.5%)
Clinical symptoms Fever <u>></u> 39C Headache Nausea Vomiting Weakness	16 (80.0%) 18 (90.0%) 12 (60.0%) 8 (40.0%) 18 (90.0%)	4 (50.0%) 5 (62.5%) 2 (25.0%) 2 (25.0%) 5 (62.5%)
Outcome Recovered Died	20 (100%) 0 (0%)	7 (87.5%) 1 (12.5%

Acknowledgment:



The study was supported in part by the project Croatian Science



Ljiljana Milasincic and Snjezana Artl for technical assistance.



International Meeting on Emerging Diseases and Surveillance, IMED 2018, 9-12 Novemer, 2018, Vienna, Austria