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Short communication

First molecular analysis of West Nile virus during the 2013 outbreak in Croatia

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ABSTRACT

This is the second subsequent year of West Nile neuroinvasive disease (WNND) outbreak in Croatia. Between July and October 2013, 22 patients presented with symptoms of WNND: all with meningitis and 18 additionally with encephalitis. In contrast to 2012, where six autochthonous infections were confirmed in eastern Croatia, the majority of this year's cases occurred in and around the city of Zagreb, where no West Nile virus infections have been observed before. Viral RNA was recovered from two patients and phylogenetic analyses revealed West Nile virus lineage 2. This represents the first molecular characterization and phylogenetic analysis of the circulating West Nile virus strain in Croatia.

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West Nile virus (WNV), family *Flaviviridae*, genus *Flavivirus* is a mosquito-borne virus belonging to the Japanese encephalitis antigenic group (Hubálek and Halouzka, 1999) and sporadic cases of human WNV infections have been observed in Europe since the 1960s (Zeller and Schuffenecker, 2004). The virus is sustained by its vector, mainly *Culex* spp. and *Aedes* spp. mosquitoes, in a rural cycle between wild birds, but can also be transmitted in an urban cycle (Hubálek and Halouzka, 1999), observed in some WNV outbreaks. Humans and horses represent dead-end hosts.

The majority of human infections remain asymptomatic, whereas 20–40% may develop an influenza like illness, termed West Nile Fever (WNF), and less than 1% West Nile neuroinvasive disease (WNND) with at least one of three major clinical syndromes: meningitis, encephalitis or flaccid paralysis (Kramer et al., 2007).

The first large outbreak of WNND on the European continent happened 1996 in Romania (Tsai et al., 1998) and was caused by a WNV lineage 1a strain (Savage et al., 1999). In recent years sporadic cases or clusters of WNV infections have been reported by several Mediterranean Countries including Croatia, Hungary, Bulgaria and Romania as well as outbreaks in Greece and neighboring Serbia (Sambri et al., 2013; West Nile fever maps, n.d.).

Serological evidence for WNV circulation in Croatia was first detected in 1980s with seroprevalence ranging from 0.28% to 4.9% in inhabitants of Dalmatia and offshore islands (Vesenjak-Hirjan, 1980; Vesenjak-Hirjan et al., 1980). Current data for the continental part showed similar seroprevalence (0.3%) and exposure of brown bears and horses (Golubić and Dobler, 2012; Madić et al., 1993, 2007).

During September 2012, seven human cases of WNND in the eastern parts of Croatia have been confirmed (Merdić et al., 2013; Pem-Novosel et al., 2013). This was expected, as an outbreak of WNV in Serbia and acute infection in horses preceded the human cases (Popović et al., 2013).

Here we present the first molecular and phylogenetic analysis of WNV causing an outbreak in Croatia between July and October 2013.

By 10th October, 22 WNND patients were admitted at the University Hospital for Infectious Diseases, Zagreb. All patients were confirmed or probable cases of WNND as defined by Croatian Public Health Authorities, which was in accordance with the Definition of the European Union (Case definitions for reporting communicable diseases, n.d.).

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Table 1
Clinical and laboratory data of West Nile neuroinvasive disease cases hospitalized at the University Hospital for Infectious Diseases "Dr. Fran Mihaljević", Zagreb ($n = 22$).

Case no.	Sex	Age	Days before hospitalization[a]	Duration of hospitalization	Department	Neuroinvasive presentation	Neurological sequelae at discharge	Glasgow Coma Score	Mechanical ventilation	WNV RNA detection in blood/CSF/urine[b]	Case classification	County of exposure
1	M	64	5	47	ICU	Encephalitis, tremor	Muscle weakness	5	Yes	−/−/−	Probable	City of Zagreb
2	M	40	6	13	ICU	Encephalitis, tremor, ataxia	Muscle weakness	15	No	+/−/−	Confirmed	City of Zagreb
3	M	61	3	14	ICU	Meningitis	–	15	No	n.a./−/n.a.	Probable	Zagreb
4	F	80	5	32	ICU	Encephalitis, tremor	Muscle weakness, tetraparesis	7	Yes	n.a./n.a./n.a.	Probable	Zagreb
5	M	61	4	30	ICU	Encephalitis	–	14	No	+/−/−	Confirmed	Zagreb
6	F	29	4	11	ICU	Encephalitis	–	14	No	n.a./n.a./n.a.	Probable	City of Zagreb
7	M	56	7	6	Ward	Meningitis	–	15	No	n.a./n.a./n.a.	Probable	Zagreb
8	F	66	3	18	Ward	Encephalitis	–	15	No	n.a./−/n.a.	Probable	Zagreb
9	M	22	3	26	ICU	Encephalitis	–	9	No	n.a./n.a./n.a.	Probable	Zagreb
10	M	28	8	16	Ward	Meningitis	–	15	No	−/n.a./−	Probable	Zagreb
11	M	68	7	8	ICU	Encephalitis, tremor	–	14	No	n.a./n.a./n.a.	Probable	Brod-Posavina
12	M	75	5	50	Ward	Encephalitis	Muscle weakness	13	No	n.a./−/n.a.	Probable	City of Zagreb
13	M	74	2	26	Ward	Encephalitis	–	14	No	−/−/−	Probable	City of Zagreb
14	M	52	8	34	ICU	Encephalitis, tremor	Bilateral n.VII palsy	7	Yes	n.a./n.a./n.a.	Probable	City of Zagreb
15	M	79	8	12	Ward	Encephalitis, tremor, ataxia	–	15	No	−/−/−	Probable	Zagreb
16	M	74	10	18	ICU	Encephalitis	–	15	No	−/−/−	Probable	Zagreb
17	M	63	6	19	Ward	Encephalitis	Muscle weakness	9	No	−/−/−	Probable	Zagreb
18	M	36	1	14	Ward	Meningitis	–	15	No	n.a./n.a./n.a.	Probable	Zagreb
19	M	68	3	15	Ward	Encephalitis, tremor	–	15	No	−/n.a./−	Probable	City of Zagreb
20	M	62	3	16	Ward	Encephalitis, ataxia	–	15	No	n.a./−/n.a.	Probable	Zagreb
21	M	73	1	14	Ward	Encephalitis	Muscle weakness	15	No	n.a./n.a./n.a.	Probable	Zagreb
22	M	59	6	18	Ward	Encephalitis, tremor	–	15	No	−/−/−	Probable	Zagreb

[a] Days of symptoms lasting before hospitalization.
[b] n.a., not available.

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