First Outbreak of West Nile Virus Neuroinvasive Disease in Humans, Croatia, 2012

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Abstract

Between September 6 and 21, 2012, seven human cases of West Nile virus (WNV) neuroinvasive infection were laboratory confirmed in Croatia. The median patient age was 62 years (range 48–77). Five patients presented with meningoencephalitis and two patients with meningoencephalitis followed by acute flaccid paralysis. Four of them had an underlying disease (hypertension). Using enzyme-linked immunosorbent assay (ELISA), WNV-specific immunoglobulin M (IgM) and IgG antibodies of low avidity were detected in six patients, whereas one showed only IgM antibodies. All samples were confirmed using plaque-reduction neutralization and micro-neutralization tests. Five patients recovered fully. Before human cases were reported, acute asymptomatic WNV infection was demonstrated by detection of IgM antibodies in sentinel horses. Moreover, an increased WNV IgG seropositivity in horses was detected in counties where human cases occurred. Adulticidal and larvicidal treatments were administered immediately in the respective places of residence. The end of the warm season contributed to the fact that there were no new cases of WNV disease recorded.

Key Words: West Nile virus—Outbreak—Croatia.

Introduction

WEST NILE VIRUS (WNV) IS A MOSQUITO-BORNE VIRUS that belongs to the Japanese encephalitis antigenic complex of the genus *Flavivirus*, family *Flaviviridae*. The virus is maintained in nature in a transmission cycle between birds and mosquitoes, mainly of the genus *Culex*, with occasional infections of humans, horses, and other mammals that are considered incidental or dead-end hosts (Kuno et al. 1998). The majority (≈ 80%) of human infections are asymptomatic. Symptomatic infections (≈ 20%) constitute primarily a mild, self-limiting febrile illness. Less than 1% of infected persons will develop neuroinvasive WNV disease, which includes meningitis, encephalitis, and acute flaccid paralysis (Hayes et al. 2005). WNV has been found to be endemic in many parts of the world, including Europe. During the last two decades, several WNV outbreaks have occurred throughout the world.

In addition to the United States, large outbreaks have been reported in Europe (Romania 1996, Russia 1999) and in the Mediterranean basin (Israel 2000; Calistri et al. 2010). Since 2010, WNV activity has increased in Europe, with outbreaks reported in Spain (Garcia-Bocanegra et al. 2011), Italy (Rizzo et al. 2012), and notably in Greece, for 3 consecutive years, starting from the summer of 2010 (Papa 2012). We are reporting the first outbreak of WNV infection in Croatia.

Materials and Methods

During September, 2012, WNV infection was confirmed by laboratory tests in seven Croatian patients. Serologic testing (WNV immunoglobulin M [IgM]/IgG, and IgG avidity) were performed at the National Reference Laboratory for Arboviruses at Croatian National Institute of Public Health using a commercial enzyme-linked immunosorbent assay (ELISA;

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TABLE 1. DEMOGRAPHIC AND CLINICAL CHARACTERISTICS AND LABORATORY RESULTS OF SEVEN PATIENTS WITH WEST NILE VIRUS NEUROINVASIVE INFECTION IN CROATIA, 2012

Case	Age	Sex	Clinical diagnosis	Underlying medical condition	WBC count ($\times 10^9/L$)	CSF protein level ^a (g/L)	CSF pleocytosis cells/mm ³	CSF WBC count ne / ly / mo (%)
1	65	M	Meningoencephalitis, acute flaccid paralysis	—	16.3	1.81	380	55/19/26
2	77	F	Meningoencephalitis	Hypertension	11.0	2.50	517	82/15/3
3	48	F	Meningoencephalitis	—	5.0	0.36	10	—/100/—
4	76	M	Meningoencephalitis	Hypertension	5.1	0.69	120	65/26/9
5	60	F	Meningoencephalitis, acute flaccid paralysis	Hypertension	9.9	0.96	41	11/42/47
6	48	F	Meningoencephalitis	—	14.1	0.82	272	16/74/19
7	65	M	Meningoencephalitis	Hypertension	3.5	0.91	17	2/75/23

^aNormal range 0.17–0.37 g/L.

WBC, white blood cells; CSF, cerebrospinal fluid; ne, neutrophils; ly, lymphocytes; mo, monocytes; M, male; F, female.

Euroimmun, Lübeck, Germany). Samples were also tested for other flaviviruses, including tick-borne encephalitis virus (TBEV) and dengue viruses (DENV) by ELISA (Euroimmun, Lübeck, Germany) and Usutu virus (USUV) by micro-neutralization test (micro-NT) to rule out cross-reactions. All samples were confirmed at the OIE Reference Laboratory for West Nile Disease, Istituto G. Caporale, Teramo, Italy by plaque-reduction neutralization test (PRNT) and micro-NT. Four samples were tested for WNV RNA using real-time reverse transcriptase-polymerase chain reaction (RT-PCR). Real-time pan-flavivirus RT-PCR and quantitative (q) RT-PCR assays for detection of WNV lineages 1 and 2 were performed in accordance with previously described protocols (Eiden et al. 2010, Johnson et al. 2010).

Results

Case reports

On September 6, the first case of neuroinvasive WNV infection in a 65-year-old man was laboratory confirmed. By September 21, six additional WNV cases were notified (Table 1). Patient disease onset occurred within a 5-week interval, from August 4 to September 10.

The median age was 62 years (range 48–77). Five patients presented with meningoencephalitis. The most common clinical signs were fever, myalgia, headache, vomiting, confusion,

restlessness, and tremor. In two patients, the disease started with clinical symptoms of meningoencephalitis followed by acute flaccid paralysis. Four patients had an underlying disease (hypertension). Five out of seven patients recovered fully. Persistent neurologic sequelae occurred in two patients. One patient (case 1) had sequelae of flaccid paralysis of the right arm and lower limbs, and the other (case 5) flaccid paralysis of the left arm and peripheral facial nerve palsy.

Laboratory results

Cerebrospinal fluid (CSF) analysis demonstrated elevated protein levels (0.36–2.5 g/L) and pleocytosis (10–517 cells/mm³) (Table 1). Serology results are presented in the Table 2. In six patients, WNV-specific IgM antibodies with low IgG avidity antibodies were detected, whereas one patient showed only IgM antibodies. WNV-neutralizing antibodies were confirmed in all samples by micro-NT and PRNT. In two patients, a four-fold increase of antibody titer in paired sera was demonstrated. The IgM responses were mainly monotypic (only two samples showed equivocal response to DENV), whereas the IgG antibodies showed cross-reactivity with DENV (four positive samples and one equivocal sample) and TBEV (three positive samples). Pan-flavivirus and the qRT-PCR for WNV lineages 1 and 2 detection were negative in all tested samples.

TABLE 2. VIROLOGY RESULTS OF SEVEN PATIENTS WITH WEST NILE VIRUS NEUROINVASIVE INFECTION IN CROATIA, 2012

Case	Serum	Real-time RT-PCR	IgM ELISA (ratio) ^a	IgG ELISA (RU/mL) ^b	IgG avidity index (AI) ^c	PRNT (titer)	Micro-NT (titer)
1	I	Neg	Pos (2.1)	Pos (102)	26%	20	20
2	I	Neg	Pos (3.3)	Pos (40)	16%	10	10
3	I	Neg	Pos (1.7)	Pos (132)	26%	10	20
4	I	Neg	Pos (3.2)	Neg (6)	—	5	10
5	I	—	Pos (3.3)	Pos (60)	11%	10	20
	II	—	Pos (2.8)	Pos (170)	—	40	80
6	I	—	Pos (4.1)	Pos (125)	16%	20	40
7	I	—	Pos (3.5)	Neg (<2)	—	5	5
	II	—	Pos (2.9)	Pos (56)	38%	20	20

^a<0.8 negative, 0.0–1.1 borderline, >1.1 positive.

^b<16 negative, 16–22 borderline, >22 positive.

^c<40% low AI, 40–60% borderline AI, >60% high AI.

IgM, immunoglobulin M; ELISA, enzyme-linked immunosorbent assay; RU, relative units; AI, avidity index; PRNT, plaque-reduction neutralization test; micro-NT, microneutralization test; Neg, negative; Pos, positive.

Discussion

In Croatia, serological evidence of WNV infection has been reported occasionally for humans (1980s–1990s) (Vesjenjak-Hirjan et al. 1980, Turković et al. 1998), European brown bears (1993) (Madić et al. 1993), and horses (2001–2002, 2010–2012) (Madić et al. 2007, Barbić et al. 2012). Clinical WNV disease in humans has not been reported so far, although clinical cases occurred in some neighboring countries (Italy and Hungary, 2008–2012) as well as other countries (Spain 2009, Romania and Greece, 2010–2012) (European Centre for Disease Control and Prevention). Furthermore, in July of 2012, the first outbreak of WNV disease occurred in Serbia with 41 laboratory-diagnosed cases and six deaths.

In September, 2012, the first outbreak of WNV neuroinvasive disease was laboratory confirmed in three Croatian counties located in the eastern part of the country, near the state border with Serbia. The first case was probably imported from Serbia where the patient resided from July 24 to July 31 (during the incubation period), whereas the others represent first autochthonous WNV cases. In the majority of patients, WNV IgG antibodies were broadly reactive with DENV and TBEV, with higher cross-reactivity between WNV and DENV. Similar results were observed during the Greek outbreak of 2010 (Papa et al. 2011).

After the WNV outbreak was confirmed, immediate vector control measures (adulticidal and larvicidal treatments) were implemented in the respective place of residence. Because environmental temperature is considered an important risk factor for WNV transmission, the end of the warm season at the beginning of October, 2012, in Croatia contributed to the fact that there were no new cases of WNV disease recorded.

Results of the WNV serosurveillance program in Croatia performed during 2012 demonstrated asymptomatic acute infection (detection of IgM antibodies) in 12 sentinel horses before human cases were reported. Moreover, an increased WNV IgG seropositivity in horses was observed in counties where human cases occurred (9.8% compared to 5.5% in 2011 and 5.0% in 2010) indicating an increased WNV circulation. These results are in accordance with the correlation between high WNV seroprevalence in horses and occurrence of human clinical cases observed in a Spanish study (García-Bocanegra et al. 2012).

In conclusion, this is the first time that clinical WNV infection was documented in humans in Croatia, in accordance with increasing WNV activity in Europe. We can expect WNV transmission in the coming years as well. Because WNV disease is an important public health concern, we should improve early detection of WNV cases to be able to control future outbreaks. Integrated WNV mosquitoes, birds, horses, and humans surveillance programs should be enhanced in future years. Public health education and preventive vector mosquito control programs, such as source reduction and larvicidal treatment with WNV surveillance, are key factors in WNV disease prevention and control.

Acknowledgments

The authors thank all laboratory personnel in Croatia and Italy for technical assistance, as well as all clinical personnel, epidemiologists, and county public health institutes personnel for their contribution to WNV outbreak control.

Author Disclosure Statement

No competing financial interests exist.

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