On 20 September 2008, the laboratory of the Regional Reference Centre for Microbiological Emergencies (Centro di Riferimento Regionale per le Emergenze Microbiologiche, CRREM) in Bologna, reported the detection of specific IgM and IgG antibodies against West Nile virus (WNV) in the serum of a female patient in her eighties who lived in a rural area between Ferrara and Bologna, Italy.

During the month of September, six confirmed and five suspected cases of WNV infection occurred in horses and were reported in Eurosurveillance on 25 September [1]. These animal cases occurred in an area in northern Italy located between the provinces of Ferrara and Bologna. Following this alert, an active surveillance programme for possible human cases of WNV meningoencephalitis was immediately started in the Emilia Romagna region. In addition, WNV has recently been identified in wild birds in the same area.

Case report

The patient showed the first symptoms, fever of over 38.0 °C and repeated vomiting episodes, on 15 September. A first diagnosis of suspected urinary tract infection was made and the patient was given oral ciprofloxacin (500 mg three times a day). The symptoms remained in spite of the therapy, and the patient was admitted to the hospital emergency room in the city of Imola on 19 September in a life-threatening condition with high fever of over 40.0 °C, vomiting, temporarily impaired consciousness, and hallucinations. The patient was not reactive and suffered from two convulsive attacks during the observation in the emergency room. Her heart rate was 99 beats per minute and her blood pressure values were 70 mmHg (minimum) and 140 mmHg (maximum), with normal arterial oxygen saturation (98%).

Due to the patient’s clinical condition it was not possible to obtain a sample of cerebrospinal fluid. The patient’s relatives reported that she had not travelled outside the small village where she has lived for the past two years. It is noteworthy that the patient’s home is located within a few kilometres from a large swamp that is home to a sizeable population of different bird species. In addition, the area is heavily infested by mosquitoes (both *Culex* spp. and *Aedes albopictus*). Six confirmed cases of WNV disease in horses have recently been reported in this area [1], and 13 birds (six crows and seven magpies) have been identified as positive for WNV by viral isolation and PCR.

Serological analysis

According to the requirements for WNV surveillance of human cases, adopted following the notification of WNV infection in horses in Ferrara (Emilia-Romagna Region), the patient’s serum samples were tested for WNV-specific antibodies using a commercial enzyme-linked immuno-sorbent assay (Euromimmun, Lübeck, Germany). The results of the serological tests indicated an acute WNV infection: IgM and IgG were both positive with a titre of 1:800 and 1:400, respectively.

On 29 September, the presence of WNV-specific antibodies was further confirmed by additional serological tests on the first samples that had been sent to the national reference centre for arboviruses at the National Institute of Health (Istituto Superiore di Sanità; ISS) and the National Institute of Infectious Diseases (Istituto Nazionale Malattie Infettive; INMI) “L. Spallanzani” in Rome. The results of those tests were the following: The indirect haemagglutination (IHA) test showed a titre of 1:1,280 (with a titre of 1:40 for tick-borne encephalitis virus (TBEV), which was expected due to the high level of immunological cross-reactivity between these two member of the Flaviviridae family); The plaque-reduction neutralisation assays (PRNT 90) showed a WNV-specific antibody titre of 1:80.

In order to assess the specificity of these results, the immunological reactivity of the patient’s sample against Japanese encephalitis virus (JEV) and TBEV was additionally assessed by immunofluorescence (Euroimmun, Lübeck, Germany), with the following results: the IgM assay for TBE and JEV was negative, whereas the IgG assay showed a titre of 1:160 for TBEV and 1:20 for JEV. The neutralisation titre against JEV was consistently <1:40. These results clearly demonstrated that the antibody response was mainly directed against WNV, thus corroborating the hypothesis of a WNV neuroinvasive infection.
A summary of all test results is presented in the Table.

Three different reverse transcription-polymerase chain reactions (RT-PCR), targeting different regions of the WNV genome (one was performed by the CRREM laboratory in Bologna and two were performed by the laboratories at the ISS and INMI) independently gave negative results, thus indicating that the patient was not at the time in the viraemic phase of WNV infection. Another RT-PCR, specific for the flavivirus genus, was also negative.

A second serum sample was obtained on 29 September. This specimen showed an increased WNV-specific antibody titre, as presented in detail in the Table, thus confirming the diagnosis of WNV infection.

Discussion
At the time of this report, the patient has almost completely recovered. She is still hospitalised as a safety and precautionary measure due to her age, but the clinical picture has improved, fever and vomiting have receded, and the patient has completely regained consciousness.

Human cases of West Nile fever in Italy have been mentioned, as personal communications, in the literature [2]. However, this is the first human case of West Nile fever that has been laboratory-confirmed and reported in Italy. It occurred at the same time as an outbreak among horses reported in same area [1]. This event highlights the necessity of a high level of epidemiological attention in order to determine the magnitude of the human outbreak, of testing organ donors from that area for WNV, and of reconsidering the previously adopted decision not to introduce any restrictions on blood donations in the area. The possibility to introduce a regular screening procedure for WNV using nucleic acid amplification techniques and serological investigation for IgM on blood donations is presently under evaluation.

Note added in proof: A second human case of WNV neuroinvasive disease has been identified by CRREM in Bologna on 3 October. It is a male patient in his late sixties who lived in an area of the province of Ferrara where WNV-positive horses and birds have recently been identified. The patient is currently suffering from symptoms of acute meningoencephalitis with high fever. To date, serum and cerebrospinal fluid samples of this patient have tested positive for IgG and IgM antibodies against WNV and two different RT-PCRs performed on the serum gave positive results. Confirmatory laboratory testing is still pending.

### Table
Serological results of the paired serum samples obtained on 19 September (1st) and 29 September (2nd) 2008

<table>
<thead>
<tr>
<th>Sample Virus</th>
<th>EIA IgM titre</th>
<th>EIA IgG titre</th>
<th>IHA titre</th>
<th>NT titre</th>
<th>IFA IgM titre</th>
<th>IFA IgG titre</th>
</tr>
</thead>
<tbody>
<tr>
<td>WNV</td>
<td>1:800</td>
<td>1:200</td>
<td>1:400</td>
<td>1:3,200</td>
<td>1:80</td>
<td>1:80</td>
</tr>
<tr>
<td>JEV</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>&lt;1:40</td>
<td>neg</td>
</tr>
<tr>
<td>TBEV</td>
<td>nd</td>
<td>nd</td>
<td>&lt;1:20</td>
<td>neg</td>
<td>neg</td>
<td>neg</td>
</tr>
</tbody>
</table>

EIA: enzyme immuno-assay; IHA: indirect haemagglutination test; NT: neutralisation test; IFA: immunofluorescence analysis
nd: not done; neg: negative (<1:10).

### References


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