A model of laboratory surveillance for neuro-arbovirosis applied during 2012 in the Emilia-Romagna region, Italy

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Abstract

Arboviruses with neuroinvasive potential are gaining more attention due to the increased number of cases of autochthonous and imported infections in the human host. Diagnosis of infection caused by these viruses in patients with central nervous system (CNS) diseases is still underestimated and these infections represent an emerging threat to public health. We describe a model suitable for the laboratory surveillance of neuro-arbovirosis that was applied in the Emilia-Romagna region, north-eastern Italy, during the 2012 summer season. One hundred and twenty cases of suspected neuroinvasive infection were tested for arboviral agents on the basis of clinical and laboratory signs and epidemiological data. The most common virus detected was Toscana virus (TOSV): anti-TOSV specific antibodies or viral components were detected in 28.3% of the cases; 79.4% of the TOSV cases were in the acute phase of infection. No cases resulted in acute phase for West Nile (WNV), Usutu (USUV), Chikungunya (CHIKV) or Dengue (DENV) virus infection. Conversely, two patients with a history of staying in a tick-borne encephalitis virus (TBEV) endemic area showed a probable TBEV infection. These results emphasize the importance of a complete and 'ready to act' laboratory diagnostic system to be implemented within the larger frame of a regional integrated surveillance system.

Keywords: Chikungunya virus, Dengue virus, Italy, laboratory surveillance, neuro-arbovirosis, tick-borne encephalitis virus, Toscana virus, Usutu virus, West nile virus

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Introduction

New infectious diseases are continuously emerging and affecting the human population; among them, vector-transmitted infections pose an increasing threat to global health and welfare [1]. Arthropod-borne viruses (arboviruses) are generally RNA viruses and can cause infections that vary from mild forms to major symptoms; symptomatic cases may develop a neuroinvasive disease [2]. Infections caused by RNA viruses with neuroinvasive potential are emerging for several reasons, including (i) the expansion of viruses to new geographical regions, (ii) spreading from animal reservoirs, and (iii) acquisition of increased neurovirulence by a previously non-neurotropic virus [2]. In general, neuroinvasive infections are an important, but likely under-diagnosed cause of neurological disease in many developing countries [3]. The constant increase of these infections in both developing and industrialized countries emphasizes the need for correct and prompt surveillance as well as for a continuous evolution of the diagnostic procedures.

In recent years, infections caused by neuroinvasive arboviruses have emerged in Italy, and in particular in the north-eastern part of the country. The first human cases of neuroinvasive West Nile virus (WNV) infection appeared in

the Emilia-Romagna region in 2008 [4], subsequently spreading to other areas of the country such as Veneto, central Italy and Sardinia [5–7]. In 2009, Usutu virus (USUV) was detected in local birds and mosquitoes in the Emilia-Romagna region [8] and has been identified as the cause of neurological disease in an immunocompromised patient [9], and as inducer of asymptomatic infections [10]. Also, Toscana virus (TOSV), a phlebovirus causing aseptic meningitis, currently circulates in the Emilia-Romagna region [11], in Tuscany [12] and in southern Italy [13]. Finally, tick-borne encephalitis virus (TBEV) is endemic in north-eastern Italy, specifically in the Provinces of Trento, Bolzano, Belluno, Pordenone, Udine, Treviso and Vicenza, where a rise in human cases has been recently reported [14].

To face the increasing circulation of arboviruses, including those causing neurological symptoms, the Regional Reference Centre for Microbiological Emergencies (CRREM) of the Unit of Clinical Microbiology at the St Orsola-Malpighi University Hospital in Bologna, Italy, has developed and is continuously improving a dedicated laboratory surveillance scheme. The first active regional programme for surveillance of arboviral infection started in summer 2007 to face an outbreak of the *Aedes albopictus*-transmitted Chikungunya virus (CHIKV) in a narrow area of the Emilia-Romagna region [15]; this programme involved clinicians, general practitioners, virologists and public health personnel. After this episode, diagnostic procedures have been continuously evolved on the basis of data obtained from an integrated surveillance system that included constant entomological, veterinary and human monitoring [16] (Emilia-Romagna region website: www.saluter.it, last accessed 24 July 2013). The Regional surveillance program has been expanded to include WNV and Dengue virus (DENV). TOSV, USUV and TBEV infection were also included in our diagnostic work flow.

The aim of this study is to present the surveillance protocol for arboviruses that has been implemented as a prototype paradigm to promptly identify emerging neuro-arbovirosis. Results obtained during the time-frame June–November 2012 are described.

Materials and Methods

Setting and samples

In accordance with the Regional surveillance program (http://www.saluter.it/, last accessed on 24 July 2013), clinical samples were sent to the CRREM laboratory during a 5-month surveillance period (June–November 2012) that overlapped with the peak period of arthropod vector activity. Samples obtained from 120 patients were sent from clinical centres located in the Emilia-Romagna region. A form accompanying the sample, including demographic information and reporting of the onset of the disease and symptoms, travel history and previous vaccination, was filled in by the physician who was responsible for the patient. The presenting clinical features were fever, aseptic meningitis and meningoencephalitis. Suggestive symptoms for DENV or CHIKV infection, such as rash, arthralgia, retro-orbital pain, haemorrhagic symptoms and/or thrombocytopenia, were also reported. Samples were collected and immediately shipped under refrigerated conditions (4°C) to the CRREM laboratory.

Molecular testing for arboviruses

Nucleic acids of arboviruses were detected in plasma/serum and/or in cerebrospinal fluid (CSF) specimens by using homemade molecular methods (see Table 1 for details). Detection of RNA was performed by real-time RT PCR for TOSV [17], TBEV [18], WNV [19,20], USUV [21] and CHIKV [22] or by a multiplex real-time RT-PCR for DENV [23].

Serological testing for arboviruses

Serological tests included detection of specific IgG and IgM in CSF and or in serum/plasma samples by commercially available kits (Table 1). Specifically, anti-TOSV, anti-CHIKV, anti-WNV, anti-TBEV and anti-DENV IgG and IgM were detected by using an indirect immunofluorescence (IIF) assay (Euroimmun, Lübeck, Germany), while DENV non-structural protein (NS1) was measured by an enzyme immunoassay (Platelia Dengue NS1 AG Kit; Biorad, Segrate, Italy).

Results

Protocol for laboratory surveillance activity

Figure 1 depicts the diagnostic work flow that was performed in all cases of suspected neuro-arbovirosis during the surveillance period. First, specific protocols for West Nile neuroinvasive disease (WNND), for TOSV and for USUV infection were implemented on CSF and/or on plasma/serum samples: this starting point was selected given the circulation of these viruses in the north-eastern part of the country. Laboratory confirmation of WNV entails molecular and serological testing [24]. Conversely, because there are no data about the persistence of TOSV in the CSF, the duration of viraemia and the timing of the appearance of antibodies following the onset of infection, molecular and serological methods were simultaneously performed to identify infection caused by this phlebovirus.