

Retrospective screening of serum and cerebrospinal fluid samples from patients with acute meningo-encephalitis does not reveal past Japanese encephalitis virus infection, Emilia Romagna, Italy, 2011

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To the editor:

A recent publication by Ravanini et al reported the detection of a Japanese encephalitis virus (JEV) RNA sequence in one pool of *Culex pipiens* mosquitoes, collected in 2010 in the province of Bologna (Emilia-Romagna region) [1]. In that study, a partial genomic sequence of 167 bp was shown to have 100% similarity to the NS5 region of the JEV genotype III genome. Confirmatory specific RT-PCR targeting the envelope, NS3 and NS5 regions of the JEV genome and attempts at virus isolation in cell culture were repeatedly negative.

Another retrospective study by Platonov et al., conducted in bird samples collected 10 years earlier, had investigated the presence of JEV RNA [2]. In particular, the authors had amplified a 215 bp fragment of the flavivirus NS5 gene in six of seven birds positive for JE group antigens in immunohistochemistry. Based on these findings, the possible introduction of JEV in a new area, and in particular in southern Europe, has been hypothesised and discussed [1-3]. We think that the detection of genomic RNA fragments (167 and 157 bp from the flavivirus NS5 gene and 552 bp from the JEV E gene in the above papers) cannot confirm the presence and circulation of JEV in Europe, and Italy in particular.

We therefore investigated the possibility of human cases of neurological infection caused by JEV in a retrospective serological study. Sera and cerebrospinal fluid (CSF) samples were obtained from 38 subjects with clinical symptoms of acute meningoencephalitis, collected in the province of Bologna between 1 January and 31 December 2011. The specimens were referred to the Regional Reference Centre for Microbiological Emergencies (Centro di Riferimento Regionale per le Emergenze Microbiologiche; CRREM)

at St. Orsola-Malpighi Hospital, Bologna, as part of the regional surveillance programme for West Nile virus infections that was started in 2009 [4]. To evaluate the presence of JEV-specific IgM or IgG antibodies, the samples were tested by a commercial indirect immunofluorescence assays (IFA, Euroimmun, Lübeck, Germany). Following the guidelines issued by the World Health Organization, the diagnosis of JEV should preferably be achieved by detection of specific IgM antibodies in the CSF, while the detection of the JEV genome (in serum, plasma, blood or CSF), or of JEV-related antigens in tissue by immunofluorescence/immunohistochemistry or virus isolation can be used in addition or as confirmatory test [5]. None of these CSF or serum samples was IgG- or IgM-positive by IFA, indicating that none of them contained specific antibodies against JEV.

In conclusion, our findings clearly indicate that no human cases of meningoencephalitis due to JEV occurred in Bologna in the months following the reported detection of a short JEV genomic sequence in *C. pipiens* pool collected in the same area [1]. A recent study conducted on different mosquito species collected between 2007 and 2010 in a larger area of the Emilia Romagna region, identified the presence of several flaviviruses closely related to JEV by RT-PCR targeting the flavivirus NS5 region; the analysis of these sequences, however, was unable to identify precisely and without doubt whether or not they corresponded to the JEV genome [6]. Moreover, 269,686 mosquitoes (of which 233,074 were *C. pipiens*) and 1,486 wild birds (418 of which were collected passively) obtained in 2011 in the context of the regional surveillance plan in Emilia Romagna, were tested with the described flavivirus-specific RT-PCR, and no JEV sequences were detected (data not shown). In addition, we are

at present still monitoring flaviviruses in mosquitoes and birds, and further amplicons belonging to the JEV genome have not been detected.

Based on these findings, the hypothesis proposed by Platonov et al. [2] of a new flavivirus, closely related to JEV, appears the most consistent. However, our findings cannot definitively exclude the possible circulation of JEV or other human pathogenic JEV-related flaviviruses in the province of Bologna and consequently, extensive human, entomological and veterinary screening with molecular techniques, will be carried out, to confirm or to rule out the possible circulation of new flaviviruses in Italy.

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