Journal of Infection (2014) 68, 290–295

BIAM British Infection Association

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Toscana virus infections: A case series from France

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Accepted 1 November 2013
Available online 16 November 2013

KEYWORDS
Phlebovirus;
Arbovirus;
Emerging;
Meningitis;
Fever

Summary Toscana virus (TOSV) is a neglected sandfly-borne pathogen in Mediterranean countries. Although discovered four decades ago, articles that describe the clinical aspects are scarce and consist mostly of case reports, with few series of cases. We studied retrospectively symptomatic TOSV infections in patients hospitalized in Marseille (France) from 2004 to 2011. Seventeen patients were classified as probable or confirmed cases. Fourteen cases (82%) occurred between June and September, and 3 cases in March, April and November. Two cases were potentially imported from Croatia and Tuscany. All patients presented with fever and neurological signs were observed such as aseptic meningitis ($n = 6$), muscular symptoms ($n = 3$), or encephalitis ($n = 4$). The outcome was always favorable. At the acute stage, anti TOSV IgM were observed in 14/17 patients, neutralization tests were positive for 3/8 patients, and RT-PCR confirmed TOSV infections in 5/8 CSF specimens.
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Introduction

Toscana virus (TOSV; *Bunyaviridae*; *Phlebovirus*) is an enveloped, single-strand negative-sense RNA virus, transmitted by phlebotomine sandflies. It was first isolated from *Phlebotomus perniciosus* and *Phlebotomus perfiliewi* collected in Italy in 1971[1] and first evidence for its human pathogenicity and neurotropism was reported 15 years after.[2,3] To date, TOSV is the only sandfly-transmitted phlebovirus that demonstrates neuro-invasiveness. In Mediterranean countries, TOSV is an emerging pathogen and a frequent cause of central nervous system (CNS) infection during the warm season. After a short incubation period, the onset is abrupt with nonspecific signs of viral

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http://dx.doi.org/10.1016/j.jinf.2013.11.006

febrile illness associated or not with CNS manifestations (aseptic meningitis (AM) or encephalitis).[4–6] Although the vast majority of cases have a favorable outcome, some severe cases of TOSV infections were described.[4,5,7–9] Human cases have been laboratory-documented in Italy, France, Spain, Cyprus, Greece, Portugal and Croatia.[4,5,10,11] Moreover, serological investigations have indicated that populations living around the Mediterranean Sea have a high risk for infection during their lifetime,[12] particularly in France.[13,14] Seroprevalence estimated from studies suggest that the incidence of TOSV is higher than estimated from clinical cases and that the occurrence of paucisymptomatic forms or mild febrile illness is frequent.

Although TOSV commonly cause CNS infections and circulate at a high level in Mediterranean population, apart from case reports, there is little clinical data consisting of case series.[6,15,16] Therefore, the clinical picture of TOSV infection remains incomplete. Criteria to distinguish confirmed and probable cases of TOSV infection are rarely enforced.[17]

Here we report a series of 15 confirmed and 2 probable clinical cases of TOSV infection recorded in the Public Hospitals of Marseille between 2004 and 2011.

Methods

Period of the study

Between August 2004 to August 2011, a total of 1896 patients with aseptic meningitis, encephalitis or neurological symptoms were tested for possible acute TOSV infection.

Definition of cases

Adapted from the Center for Disease Control and Prevention (CDC) 2011 case definition criteria for arbovirus infection,[17] the definition of TOSV cases was the following.

A confirmed case was defined as a clinically compatible case of TOSV infection (fever >38 °C) AND recent presence of the patient in an endemic/epidemic region during the period of activity of sandflies (March to end of October) AND absence of a more likely clinical explanation together with at least one laboratory criteria for a confirmed case:

- (C1) isolation of virus from, or demonstration of specific nucleic acid in blood or CSF, OR
- (C2) four-fold or greater change in virus-specific quantitative antibody titers in paired sera, OR
- (C3) virus-specific IgM antibodies in serum with confirmatory virus-specific neutralizing antibodies in the same or a later specimen, OR
- (C4) virus-specific IgM antibodies in CSF and a negative result for other IgM antibodies in CSF for arboviruses known to be endemic in the region where exposure occurred.

A probable case was a case that meets the above clinical criteria and the laboratory criteria for a probable case as virus-specific IgM antibodies in CSF or serum but with no other testing (C5). The presence of meningitis, encephalitis, acute flaccid paralysis, or other acute signs of central or peripheral neurologic dysfunction defines a neuro-invasive case.

Diagnostic assays

Virus isolation

To attempt virus isolation, when it was possible, 10 μl of CSF of patients were inoculated onto Vero cells and then incubated during 6 days at 37 °C with 5% CO2. A volume of 1 ml of supernatant was then used to inoculate a new flask of cells, incubated until the appearance of a clear cytopathic effect, as previously described.[18]

Molecular diagnosis

Virological tests were performed in the Virology laboratory of the Public Hospitals of Marseille. A 200-μL volume of CSF or whole blood was used for total nucleic acid purification with the EZ1 Virus Mini kit onto the BioRobot EZ1 (both from Qiagen) into a 90 μL elution volume of which 10 μL were used for cDNA synthesis using the TaqMan® Reverse Transcription Reagents (Life Technologies) as recommended by the manufacturer. A 10 μL volume of cDNA was used for real-time PCR assay specific for Toscana virus.[19]

Serology screening

The presence of IgG and IgM specific for Toscana virus was studied by indirect immunofluorescence (IIF) assay, as previously described[20,21] with minor modifications. Briefly, equal amounts of infected and uninfected Vero cells were mixed together and spotted onto 2-well glass slides through a 3-min cytospin-based centrifugation at 900 rpm. Samples were tested at 1:20 dilution in phosphate-buffered saline. The presence of uninfected Vero cells allowed to detect non specific fluorescence.

Serology confirmation

The virus microneutralisation (MN) assay for TOSV was performed as previously described[22] with minor modifications. Briefly, MN assay was performed in 96-well microtitre plates using Vero cells (ATCC CCL81). Two-fold serial dilutions from 1:10 to 1:1280 were prepared for each serum and a volume of 50 μL was pipeted into 96-well plates, using an epMotion 5075 working station (Eppendorf). The Toscana virus strain MRS2010-4319501 (GenBank accession nos KC776214—KC776216KC776214KC776215KC776216), isolated from a human case of meningitis in Southeastern France in 2010,[18] was titrated in Vero cells. A volume of 50 μL containing 100 $TCID_{50}$ was added into each well except for the controls that consisted of PBS. The plate containing 100 $TCID_{50}$ of virus and 1:10 to 1:1280 dilutions of serum was incubated at 37 °C for one hour. Then, a 50 μL suspension of Vero cells containing approximately $2{\cdot}10^5$ cells in 5% foetal bovine serum was added to each well, and incubated at 37 °C in presence of 5% CO_2. After 5 days, the microplates were read under an inverted microscope, and the presence or absence of cytopathic effect was noted. MN assay was performed retrospectively on samples from the 16 patients hospitalized during the 2004—2010 period, and was prospectively performed on samples from the single 2011 patient (P6, Table 1).

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