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Reference


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Toscano virus meningitis case in Switzerland: an example of the ezVIR bioinformatics pipeline utility for the identification of emerging viruses

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Abstract

Toscano virus (TOSV) represents a frequent cause of viral meningitis in the Mediterranean Basin that remains neglected in neighbouring countries. We report a documented TOSV meningitis case in a traveller returning from Tuscany to Switzerland. While routine serological and PCR assays could not discriminate between TOSV and Sandfly fever Naples virus infection, a high-throughput sequencing performed directly on the cerebrospinal fluid specimen and analysed with the ezVIR pipeline provided an unequivocal viral diagnostic. TOSV could be unequivocally considered as the aetiological agent, proving the potential of ezVIR to improve standard diagnostics in cases of infection with uncommon or emerging viruses.

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Introduction

Toscano virus (TOSV, Phlebovirus) causes human infections via bites from infected phlebotomes [1], leading to central nervous system (CNS) infections in a minority of cases. The prevalence of human infection is closely linked to the Mediterranean distribution of the vectors during the warm season [2,3]. Despite documented evidence of TOSV presence in neighbouring countries, its investigation remains neglected in most routine laboratories in Europe. TOSV infection is usually diagnosed by detection of IgM, although cross-reactivities of antibodies targeting TOSV and Sandfly fever Naples virus (SFNV, Phlebovirus) can occur [4,5]. Additionally, diagnostics based on RT-PCR assays are problematic because of the short period of viraemia and the low viral load in the cerebrospinal fluid (CSF) during the acute phase [6–8].

Here we report a documented case of TOSV meningitis in a traveller returning from Tuscany to Switzerland. The patient is a 61-year-old healthy man admitted in July 2012 to the Emergency Department of the University Hospitals of Geneva, Switzerland, presenting headache, nausea, photophobia and phonophobia, and fever up to 38.3°C. Except for nuchal rigidity, the neurological examination and the cerebral computed tomography were normal. Five days earlier the patient had returned from a 3-week vacation in Tuscany, Italy, where he noticed sandfly bites. His CSF analysis revealed 134 leucocytes/mm³; including 45% lymphocytes, 43% monocytes and 12% neutrophils. Direct Gram stain examination was negative, glucose level was 3 mmol/L (for a 6.7 mmol/L glycaemia) and the protein level was 0.78 g/L.

Investigations using PCR for herpes simplex virus types 1 and 2, varicella zoster virus, parechovirus and enterovirus were negative in the CSF specimen, and serological testing showed no evidence of infection with West Nile virus. The patient was put on intravenous amoxicillin plus ceftriaxone. Antiviral therapy was not introduced. Bacterial cultures remained negative.

A real-time RT-PCR for TOSV was positive in CSF whereas serum taken 2 days later was TOSV negative. The real-time RT-
PCR used for TOSV detection was an in-house version (see Supporting information, Table S1) of a previously described method [9]. Primers and probe were adapted to ensure the detection of all TOSV sequences available in GenBank, with the consequence that other members of the same genus like SFNV species were also detected. Results of indirect immunofluorescent assays (Sandfly_fever_virus_IgM/IgG mosaic; EuroImmun, Lübeck, Germany) on the serum sample were IgM and IgG positive for TOSV and SFNV for dilutions 1:10, 1:100 and 1:1000. The patient recovered within 5 days.

Taken together, routine serological and PCR assays confirmed the presence of a phlebovirus genus member in this meningitis case, but the species identification remained inconclusive. Although TOSV was the most likely candidate, an SFNV infection could not be ruled out. Due to the low viral load detected in the positive CSF specimen, both inoculation on susceptible cell lines such as Vero, SW13, BHK-21 or CV-I and virus typing by classical PCR-based sequencing approaches could not be considered. Furthermore, cloning real-time RT-PCR amplicons was not successful. Hence, high-throughput

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**FIG. 1.** ezVR analysis phase-1 report. Plot depicts: (1) the detection of Toscana virus (TOSV) L- and S-segment sequences in the cerebrospinal fluid (CSF) specimen, (2) for each detected TOSV segment: the per cent genome coverage (x-axis), the maximum coverage depth (y-axis), the total covered length in base pairs (area of the coloured dot) and the genome size group (grey outer ring). More details, including the cross-referenced segment IDs, are contained in the corresponding table.

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sequencing (HTS) technology could be considered in this situation.

We recently developed a bioinformatic pipeline (ezVIR) that processes HTS data from any of the standard platforms, screens all known human viruses, and provides easy to interpret virus diagnostic results [10]. ezVIR provides HTS results in two phases: phase 1 provides a rapid identification of the best-scoring viruses present in the specimen analysed and the optional phase-2 stage provides both virus typing and genome coverage statistics for any viruses detected in phase 1. Importantly, the pipeline is designed to not only detect viruses most often analysed in daily practice, but also any documented human virus including uncommon and emerging viruses. Therefore, to provide a complete viral diagnostic result for the case presented here, HTS analysis (paired-end sequencing using the 100-bp protocol with indexing on a HiSeq 2500; Illumina, San Diego, CA, USA) was performed directly on the CSF specimen and analysed using the ezVIR pipeline as previously described [10]. The ezVIR phase-1 report detected the presence of TOSV in the CSF specimen analysed (Fig. 1). Reads mapping specifically to the L- and S-segments of TOSV genome were observed, but no reads mapped to SFNV reference sequences. Therefore, TOSV can be unequivocally considered as the aetiological agent responsible for the meningitis case depicted here, ruling out a potential SFNV infection. Notably, the ezVIR database consists of all complete mammalian and avian virus genome sequences publicly available (>11 000 full-length genome sequences). While the ezVIR phase-1 and phase-2 reports suggest that the specimen contains TOSV genotype A (Fig. 1, corresponding table), the phase-2 genotyping analysis is based on the limited number and diversity of TOSV sequences currently available (36 sequences), and therefore should be considered with caution.

As mentioned above, in Mediterranean countries TOSV represents a frequent cause of CNS infection during the summer season, whereas beyond the endemic area such infections remain largely neglected. In Switzerland only two cases of TOSV meningitis were previously described, both from travellers returning from Elba Island (Table 1). Importantly, although serological analyses gave positive IgM titres in both cases, only case 2 could be confirmed by a positive real-time RT-PCR (Table 1), enabling clinicians to rule out any potential serological cross-reactivities. The TOSV infection documented in this study confirms the importance for clinicians to be aware of the possibility of infection with this emerging virus in patients returning from endemic areas, especially from Italy [4,11,12], Spain [13,14], France [2,15], Portugal [16], Croatia, Slovenia, Turkey [17], Greece, Algeria, Malta, Cyprus and Tunisia [2,5,6,18–20].

Although sensitivity and specificity represent parameters of utmost importance for the reliability of interpreting diagnostic assays in routine practice, both are frequently not optimal for tests dedicated to rare or emerging viruses. Indeed, for such viruses, molecular or serological assays are frequently either not available or not specifically adapted to novel identified circulating strains/genotypes, frequently leading to inconclusive interpretations. This TOSV infection perfectly exemplifies this lack of specific diagnostic assays for routine screening. Therefore, analysis of HTS data using pipelines such as ezVIR circumvents the need to establish and validate an assay that is time consuming and requires frequent updates and quality checks according to the novel sequences available. Here, the use of the ezVIR pipeline enabled TOSV detection directly from the CSF specimen, demonstrating the capacity of this pipeline to rapidly provide an unequivocal result interpretation whereas all those obtained by classical serological and molecular routine assays remained incomplete or inconclusive. These results and the ease of their interpretation prove the utility and the potential of ezVIR to improve standard diagnostics in cases of infections with emerging viruses.

<table>
<thead>
<tr>
<th>Case</th>
<th>Destination</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Incubation (days)</th>
<th>Toscana virus serology IgM IgG</th>
<th>Toscana virus PCR (in CSF)</th>
<th>Outcome</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Elba island</td>
<td>~20</td>
<td>M</td>
<td>10–15</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>Recovered within 3 days</td>
</tr>
<tr>
<td>2</td>
<td>Elba island</td>
<td>17</td>
<td>M</td>
<td>14</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Recovered within 5 days</td>
</tr>
<tr>
<td>3</td>
<td>Tuscany</td>
<td>61</td>
<td>M</td>
<td>6–21</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Recovered within 5 days</td>
</tr>
</tbody>
</table>

### Transparency declaration

No conflict of interest

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cmi.2014.11.010.

References