Reviewer's report

Title: Involvement of Toscana virus in cases of meningitis in Tunisia

Version: 2
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Reviewer: Koray Ergunay

Reviewer's report:

The manuscript describes investigations on the epidemiology of Toscana virus (TOSV) and other phleboviruses in Tunisia, via virus detection in central nervous system infections and field-collected sandflies throughout the country. Although a number of previous reports clearly indicate TOSV circulation in the country, the study addresses the issue directly and provides evidence from both human subjects and vectors with sufficient numbers. Thus, the overall findings contribute to the current data on phlebovirus epidemiology. However, several issues that require explanation, profound discussion and/or extensive re-writing have been observed in the manuscript.

Major Compulsory Revisions

1. (Material and Methods, serological investigation, paragraph 1): The authors must provide information on the ELISA system used for TOSV serology in the text. Is the method optimized for both serum and cerebrospinal fluid (CSF)? What is the CSF dilution employed? Does the method incorporate native or recombinant antigens? What are the sensitivity/specificity values? An approach for test interpretation is also given in the text. Is this compatible with the manufacturer's instructions?

2. Significant variations are reported for sensitivity-specificity and agreement for commercial assays for TOSV serology (Ergunay et al, Vector Borne Zoonotic Dis. 2011). The issue of false positive and negative result and possible cross-reactions with the Punique virus should be included in the discussion. The authors should not call their ELISA findings as "TOSV specific", since the results were not confirmed via virus neutralization.

3. Associated with the comment above, it is more appropriate to consider all serology results lacking specificity confirmation via virus neutralization assay in serum samples as probable infections. Since a concominant serum sample is missing in cases with available CSF, a reactive ELISA result representing a spillover of immunoglobulins due to a damaged blood-brain barrier can not be excluded. This posint must be considered in CSF samples with viral RNA and IgM detection. A clear definition of confirmed and probable cases and related assay results should be provided in the text. Have the authors interpreted TOSV IgM+IgG ELISA positive cases as acute TOSV infections as well? Specific TOSV IgM has been demonstrated to persist in asymptomatic blood donors (Ergunay et al, Zoonoses Public Health. 2012). Please provide a reference for statements on the timing of TOSV IgM-IgG response in the first paragraph of the discussion.
section.

4. A list of detailed clinical manifestations and laboratory findings should be provided in confirmed cases (viral RNA positive) and probable cases as a table.

5. Has Sandfly Fever Sicilian virus, detected in one patient CSF sample, been considered the causative agent? Since this is a rare finding, it should be elaborated thoroughly in the text, as similar phleboviruses have infrequently been implicated in central nervous system infections (Becker et al, Klin Padiatr, 1997; Ergunay et al, J Clin Virol, 2012). The authors should also discuss the diagnosis of the previously reported case from Tunisia.

6. A discrepancy in the explained methodology (Material and Methods, pooling and RNA extraction, paragraph 1) for sandfly processing is evident (Results. Detection of TOSV in sandflies). Please clearly define how many specimens were included in virus detection and morphological identification. Table 2 provides insufficient data. This table should be revised to include: - precise numbers of female and male sandflies and species distribution in each sampling region, - precise number and species of pools evaluated for phlebovirus RNA.

7. For the comparison of nucleic acid sequences, an identity matrix of the detected TOSV sequences with prominent TOSV strains, preferably including all known genotypes in the public databases will be more useful than BLAST searches.

8. Figure 2 must include the detected Sicilian and Punique virus sequences as well as other phleboviruses, Punique, Sicilian, Naples, Utique etc. to demonstrate phylogenetic relatedness of the observed sequences as well as all three genotypes/lineages of TOSV.

9. Table 1 must be modified to accommodate data from sera as well. A cross-table may not be required.

10. Table 3 is not necessary and the accession numbers of CSF and sandfly-originated sequences can be provided in the text.

11. The conclusion section is not related to the current findings of the authors but provides general statements about the emergence/resurgence of arthropod-borne viral infections. Please include TOSV detection in sandflies and other prominent findings of the study.

- Minor Essential Revisions

1. The title of the manuscript does not fully represent the contents and provides the impression of a clinical study only. The authors are recommended to modify the title to accommodate the field survey as well.

2. Although the text can be interpreted sufficiently, it will surely benefit from the corrections provided by a native English speaker. (for example: (Phylogenetic analysis, last sentence): "...due to its small nucleotides and internal mutations.")

3. (Material and Methods, sample collection, paragraph 2, lines 102-105): the sentences explaining which assays were performed in samples must be removed to be included in the results section.
4. Producers of commercial products employed in the study is not indicated homogenously in the text. Please check all commercial product information, including computer softwares.

5. Virus isolation efforts must be quoted in the Results section.

6. Several typing errors were observed, please check the entire document.

- Discretionary Revisions

1. (Background, paragraph 4): The sentence describing the aim of the study is not accurate (as sera is also included). Please modify the sentence with more general statements, as the details will be explained in the following sections.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests