Author’s response to reviews

Title: Meningitis and Epididymitis caused by Toscana Virus Infection Imported to Switzerland Diagnosed by Metagenomic Sequencing: a case report

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Reviewer 1

# First of all, it was a pleasure for me to read your article. It is a very interesting case report.

Reply: We thank the reviewer a lot for her nice comments and are happy that our case report is of high interest.

# Line 41-Epididymitis was treated with ciprofloxacin and neurological symptoms recovered spontaneously after 5 days- if etiology of epididymitis was viral one, ciprofloxacin administration based on what considerations was performed?
Reply: Normally, epididymitis is caused by Gram-negative bacteria. Therefore, ciprofloxacin was administered according to local antibiotic guidelines. We don't know if epididymitis was associated with meningitis, it might be also a co-incidence of two different infectious diseases. However, since we found three other cases with initial presentation of epididymitis (references 8 - 10), we cannot exclude an association. Up to know, no sexual transmission is known in TOSV infection.

# Line 88- Finally, fungal, bacterial and mycobacterial cultures of CSF remained negative-certainly the results of these cultures, at least for mycobacteria were late, not during hospitalization period.

Reply: We agree on that. We reworded the sentence so that it reads now: “Since all tests including conventional cultures after 4 days were unremarkable, unbiased metagenomic virus sequencing of the CSF and serum was performed. Finally, fungal, bacterial and mycobacterial cultures of CSF remained negative with prolonged cultivation time” (line 87).

# Line 99- (over 10 days, i.v. ceftriaxone, switched to p.o. ciprofloxacin upon discharge)- in the abstract treatment with ceftriaxone is not included. I deduce that the treatment was initiated and continued for 10 days, that means the patient has been receiving antibiotic throughout the meningocerebral impairment for all the period - were bacterial aetiologies not excluded?

Reply: It is correct that we initially treated meningitis and epididymitis with ceftriaxone. We recognize that rewording of the abstract should be done for a better understanding. It reads now as: “Meningitis was initially treated with ceftriaxone and neurological symptoms recovered spontaneously after 5 days” (line 41).

# What about the CSF cellularity - a lymphocytic one, CRP, leukocytes?

Reply: We describe the CSF abnormality on line 78 onwards: “A cerebrospinal fluid (CSF) examination revealed a pleocytosis of 53 cells/μl consisting primarily of lymphocytes (70%) and monocytes/macrophages (23%), normal glucose, increased lactate (3.3 mmol/l) and moderately increased total protein levels (0.821 g/l).” In summary, the patient suffered from a lymphocytic meningitis.
# Line- 138-140: Typical clinical findings of TOSV infections are a headache, fever, rash and gastrointestinal symptoms such as nausea and vomiting- I consider that this are not typical manifestations of an etiology, but of an meningitis.

Reply: We agree on that and reworded the sentence to: “Typical clinical findings of TOSV infections are a headache, fever, rash and gastrointestinal symptoms such as nausea and vomiting [7], which can also be found in meningitis caused by other pathogens“ (line 142).

# The conclusions are missing. The case itself does not bring anything spectacular, rather the laboratory part is quite detailed. The etiology of meningitis is important for Switzerland as risks associated with international tourism.

Reply: In our opinion, there are four main conclusions of this case:

- This is only the fourth case of TOSV imported into Switzerland. With increasing travel histories of patients, physicians should be aware of imported TOSV as the agent for viral meningitis and meningoencephalitis.
- Epididymitis-orchitis, as present in our patient, has already been described in a few other cases as an unusual manifestation of TOSV infection.
- The identified isolate belongs to the TOSV lineage B, although in Italy so far only lineage A has been reported. For the relevance of this finding, see reply below to reviewer 2.
- A rapid identification of a viral etiology has direct consequences for antibiotic treatment. Metagenomic sequencing thus allows for detection of rare or unexpected viral infections otherwise not accounted for in routine diagnostics.

We tried to highlight these conclusions better in the main text on line 156, “… co-circulation of two genotypes has been shown previously in southeastern France close to the origin of the isolate in this study“, and line 164 “A rapid identification of a viral etiology may have direct consequences for treatment and limit the unnecessary prescription of antibiotics”.

Reviewer 2
The manuscript is well written as the experimental design. I have no criticized to do, only some minor commentaries and recommendations.

Reply: We thank the reviewer for her nice comments about our manuscript.

Cite in the text the methodology used for the detection of IgG and IgM.

Reply: Serology was performed with the "Sandfly fever virus Mosaic 1 types Sicilian, Naples, Toscana, Cyprus IgG and IgM assay" from EuroImmun, Luebeck, Germany. This was described in the additional methods, but as the reviewer suggested, we added it to the main text (line 96).

As described in the literature, the genotype A is the main or the only genotype circulating in Italy (Baggieri et al. 2015 doi: 10.1016/j.virusres.2015.01.013). This imported case (from Italy) was caused by genotype B. What is the relevance of finding a different genotype that is potentially circulating in Italy?

Reply: The epidemiological relevance of this finding is that TOSV lineage A may not be the only lineage circulating in Italy. So far, most isolates sequenced from Italy were from the Tuscany region (Baggieri et al). Therefore, they may not reflect the lineage distribution of entire Italy. The isolate described in this study originates from Liguria, which borders France. As TOSV lineage B has been described in France, co-circulation in this region is not an unlikely scenario. Further studies will be necessary to define co-circulation of lineages A and B in northwestern Italy.

As for the clinical relevance, we are not aware of any reports that show a higher pathogenicity of one of the lineages, so there is most probably no clinical relevance. With regards to the associated epididymitis, one of the cases was infected with a lineage A TOSV (Baldelli et al.), the lineages of the others are not known.

We added a comment on the clinical relevance to the manuscript (“To our knowledge, no difference in clinical presentation has been described between the two lineages“, line 157) and included the reference for Baggieri et al. 2015.