Reviewer's report

Title: Evidence of West Nile Virus Infection in Nepal

Version: 3
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Reviewer: Khin Myint

Reviewer's report:

An interesting report on the emergence of West Nile virus in Nepal where Japanese encephalitis is endemic. A few points need to be considered for clarification and improvement as well as seeing to some typing errors.

Minor revisions:

1. Please correct inconsistencies; Lines 40-41 (12 out of 2041 were tested positive by RT-PCR) was contrary to Lines 96-97 (only 2 out of 14 samples showed the presence of WNV RNA by nested RT-PCR…)

2. Line 75/ Line 142 – was this an IgM antibody ELISA? Recommend to briefly update the kit or reference. It would be helpful if the authors briefly comment on the cross-reactivity of flaviviruses.

3. Line 143 “suggesting the clinical specimens were collected during the decline stage of WNV viremia”- Was there any data on when the specimens were collected after onset of illness (fever) for these two patients?

4. The second case had a co-infection with dengue (lines 121-122). Please clarify on how this was diagnosed (ELISA and/or PCR). A few comments on the co-infection of flaviviruses in the discussion would be interesting.

5. Clinical presentation (page 5) – would be advisable to mention any recent travel history to where WNV is reported (e.g. India) and to emphasize that both patients came from urban communities.

6. Nested RT-PCR method is described on lines (77-78, 81-82, 97-98). Might be helpful to describe the strain of WNV positive control to rule out contamination. What is the homology of the positive control to the 2 samples VIROAF73 and VIROAF74.

7. Clinical presentation (Page 5) – Suggest to include relevant laboratory findings (e.g. thrombocytopenia) and clinical outcomes. Any relevance for including the immunization history? Also the clinical findings were non-specific and could be summarized in one comprehensive paragraph. Rather than the exact dates, it might be more useful to mention that the patients presented xx days after the onset of illness.

8. The evidence of more than 2 lineage of WNV (Lines 158-160) has been reported before (e.g. Bakonyi et al 2005, Bondre et al 2007, Ivov et al 2004).
Recommend to bring this up in the Discussion in relation to your findings.

9. Concluding remarks (lines 166-168) – It is true that with a wide spectrum of clinical illness, WNV could be one cause of febrile illness. Its presence also indicate that it might be useful to exclude WNV in CNS infections (e.g. encephalitis, acute flaccid paralysis, etc) especially since another flavivirus (Japanese encephalitis) is prevalent in the Terai area of Nepal and could cause cross-reactivity in serological tests.

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

**Quality of written English:** Acceptable

**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.