Reviewer’s report

Title: Characterisation of circulating, clinical Influenza isolates from Bali, Indonesia: preliminary report from the BaliMEI project.

Version: 1 Date: 19 Dec 2016

Reviewer: Andrey Komissarov

Reviewer's report:

line 90: Only 64 out of 95 samples has concurrent epidemiological information. Were there any inclusion criteria for this study? Were WHO definitions of SARI and/or ILI used? What PCR system was used for influenza virus detection?

line 100: Illumina Nextera XT Kit uses DNA as an input. In case of influenza virus you need to specify whether you have used cDNA after reverse transcription or amplicons after RT-PCR. Consider providing details of your protocol.

line 101: "The extracts were quantified using the Illumina MiSeq platform" - what does it mean?
line 102: "Genome assembly <…> was performed at Indonesia" Is it important to indicate the state?
lines 102-105: it is better to move bioinformatics from "Sample sequencing" to "Sequence de novo assembly" or "Phylogenetic analysis" sections.
lines 108-115: Are sequenced genomes available in GISAID or GenBank? Please provide accession numbers. It is highly recommended to submit sequences to public databases. Making sequences not available to scientific community precludes independent check of reported results.
line 126: Why did you choose GTR+Γ4 substitution model? What algorithms did you use for model selection?
line 131: "mean age was 14,65+-15,56 years old" - does it make sense to provide such figure?
line 134: "influenza A was mostly occurred in children age 0-4 yo…" - it is not clear how this conclusion was drawn
Identification of seasonal H1N1 virus in human after 2009 is an extraordinary event that should require thorough investigation and independent proof. WHO recommends to send specimens suspected to be positive for sH1N1 immediately to WHO Collaborating Centres for confirmation.

"sufficient concentration and quality" - how quality was assessed? what concentration threshold for inclusion in NGS run was used?

It is better to move NGS run characteristics (e.g. average depth, coverage, etc.) to "Methods" section.

results of phylogenetic analysis should be significantly revised and extended. What phylogenetic clades do sequenced strains belong to? What genes were used for phylogenetic analysis? What influenza reference and vaccine strains were used in phylogenetic analysis?

"lack of subsequent transmission" - what phylogenetic methods were used to assess transmission of strains between different geographical regions? Did you make any phylogeography? Conclusions are not supported by data shown.

"Bali, 131 Indonesia" - what does it mean?

Can this conclusion be made using such a small sample size?

It is not clear how the lack of preselection by Ct value can improve NGS output?

Table 1: what "p+" sign means?

**Are the methods appropriate and well described?**

If not, please specify what is required in your comments to the authors.

No

**Does the work include the necessary controls?**

If not, please specify which controls are required in your comments to the authors.

No

**Are the conclusions drawn adequately supported by the data shown?**

If not, please explain in your comments to the authors.

No
Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?

If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

Not relevant to this manuscript

**Quality of written English**

Please indicate the quality of language in the manuscript:

- Needs some language corrections before being published

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