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Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 12293-review.doc).

Title: Reverse genetics; unlocking the secrets of negative sense RNA viral pathogens

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The manuscript has been improved according to the suggestions of reviewers:

1. **Format has been updated**

2. **Revisions have been made according to the suggestions of the reviewers**

Reviewer 00504045

The authors have included table 1 to summarize virulence factors and their roles in pathogenesis, which were described in the REVERSE GENETICS AND PATHOGENICITY FACTOR DISCOVERY section. Table 1 has been referred to in the text; line 189, 232, 255, 258, 270, 271 and 328.

The CONCLUSIONS AND FUTURE DIRECTIONS section has been rewritten and expanded to cohesively outline the major topic; reverse genetics.

Line 394 "CONCLUSIONS AND FUTURE DIRECTIONS

Reverse genetic technologies have proven critical to study the contribution of viral genetic factors to disease severity by enabling production of well-defined, recombinant negative-sense RNA viruses, such as a mutant and wildtype viruses, which can be compared for the purpose of identifying chief virulence determinants in the context of host-pathogen systems. For several negative-sense RNA viruses effective rescue methods have been developed, which may be dependent upon either T7 or PolI and II transcriptional units. Furthermore, inclusion of polymerase proteins or 5′ and 3′ cleavage sequences for correct vRNA processing may also be necessary for rescue, although these conditions are optimized for each virus and minigenome assays have proved useful for this purpose.

Recombinant viruses, however, are produced by selection of a consensus sequence that forms the basis of the infectious clone and therefore recombinant viruses are likely to constitute only the dominant viral species of a potentially diverse natural virus population. Reduction and alteration in viral heterogeneity, as a consequence of reverse genetics, is a limitation not often taken into account in the context of pathogenesis studies. However, with the advent of next generation sequence technologies for thorough characterization of virus populations we stand in good stead to gather a better grasp of viral heterogeneity in a field isolate and molecular biologists may be capable of recapitulating diverse viral populations via reverse genetics. Recent technologies such as Gibson cloning[130] and barcoding virus populations[131] are likely to enable researchers to produce heterogeneous virus populations that can be studied for characterization of the pathogenic potential of diverse viral populations, with a particular focus on the importance of subdominant viruses for severe disease outcomes. Despite this

limitation, reverse genetics enables production of viruses that may be utilized for various future applications such as live-attenuated vaccines, mapping neural pathways in the brain, oncolytic virus production and delivery of microRNAs as a therapy for viral infections."

Minor aspects have been corrected: 1. Line 349, reverse engineered was amended to "recombinant viruses were produced"; Line 368 phentoype was amended to phenotype; 'Single stranded' and 'negative sense' were hyphenated throughout the document; viral nomenclature was revised to represent standard terminology ie. Line 85 (Respiratory syncytial virus), Line 100 (Borna disease virus), when used as a proper noun influenza virus has been amended to "Influenza virus"; line 132 (Hepatitis delta virus), Line 120 (Newcastle disease virus); Line 121 (Vesicular stomatitis virus), Line 221, 224, 231, 239 (Ebola virus isolates).

Reviewer 00503935
C, V and W proteins are viral accessory proteins and are expressed in a non-abbreviated form. Their roles during infection are described in lines 327-330.

pCAGGS is a vector containing the CAG promoter which is made up of the CMV early enhancer fused to a chicken β actin promoter. It was used as an example in this sentence. As pCAGGS is referred to in Fig 1A the pCAGGS vector has been described here as well as the CAG promoter.

Line 73 "Eukaryotic expression vectors such as pCAGGS contain strong promoters such as CAG, chicken β actin fused to a cytomegalovirus enhancer, and transient transfection of polymerase constructs promote sufficient viral protein expression to elucidate the minimum number of viral proteins required for reporter expression."

-ve RNA is an abbreviated form of negative-sense RNA that has been added to the figure legend and altered in Fig 1a for consistency between 1a and 1b.

3. References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Clinical Infectious Diseases*.

Sincerely yours,

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