Reviewer's report

Title: Highly pathogenic influenza A virus (H5N1) can be transmitted in ferrets by transfusion

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Reviewer: Troy Sutton

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Reviewers Report:
Highly pathogenic influenza A virus (H5N1) can be transmitted in ferrets by transfusion.

Wang et al., report on transmission of highly pathogenic avian influenza H5N1 via transfusion in ferrets. They demonstrate that transfusion of blood from infected ferrets results in infection of naïve donor animals. Furthermore, the results indicate that transfusion earlier during infection in the donor appears to result in higher levels of viremia. The manuscript has been significantly improved from earlier versions and the data tables are clear and much easier to follow. However, some additional changes are required prior to publication. These changes are outlined below:

Major Compulsory Revisions:
1) The representation of data in Figure 1 is misleading. While the authors are trying to show group differences, it is not appropriate to group the data from ferrets infected on different days in panels B, C, and D. The grouping of data in panel A is correct because these ferrets were infected at the same time. However, in panel B, the data needs to be divided. Both lines represent the combined data from animals infected with blood from donor ferrets on different days post-infection. These are different groups and therefore need to be plotted separately. Furthermore, statistical analysis on the current groups is not appropriate. Similarly, in panel C, several of the ferrets in the high dose group died and, therefore, all the weight loss data cannot be expressed as an average. The data as it is currently plotted suggests all of the ferrets survived. Similarly, in panel D, the data represents group averages, but the groups, especially in the high dose group, are being reduced in size by mortality over time. It might be more appropriate to give temperature data as a maximal increase above baseline and present this in a table.

Minor Essential Revisions:
1) Throughout the manuscript including the abstract, the use of percentages to report data can be misleading. Often there are small numbers of animals, for example, in Figure 2C, using 1/1 as 100% is not appropriate, please also modify the text on line 258 to reflect this as well. Thus it is more appropriate to express the results as 2/12 (16.67%) or 1/12 (8%).
2) Please use proper scientific notation to denote virus dose. 10 x 10+2.6 should be denoted as 1 x 10+3.6. Furthermore, I would suggest that you convert 1 x 10+2.6 TCID50 to 398 or 400 TCID50 or 4 x 10+2 TCID50. This would assist the reader in relating the work to other publications.

3) Line 77-79 of the introduction needs to be modified. H5N1 has not successfully spread into humans. It sporadically or occasionally infects humans. There is no stable H5 lineage in humans.

4) Line 87, please indicate that the virus is highly pathogenic to chickens. In the introduction it is often unclear if you are referring to birds or mammals with respect to pathogenicity. The highly pathogenic designation is for chickens.

5) Methods section line 117, please indicate the virus strain name in full.

6) Lines 161 and 171, please clarify if this is BSL3 or BSL3 enhanced.

7) Line 165, please change the capitalization of Ketamine/Xylazine to small font.

8) Line 205, please remove p<0.01 is very significant.

9) Line 211, please modify. The % mortality graph does not indicate a “rate” of mortality.

10) Lines 309-311 in the discussion introduces new data. This data should be mentioned in the results.

11) Lines 337 to 340 need to be modified. The authors indicate that H5N1 may have broader tissue tropism, but the results do not support this claim. The findings do not provide evidence of replication in blood cells. As indicated in the introduction, H5N1 already infects multiple tissues, and others have reported infection of ferrets infected via inoculation into the stomach. It is more appropriate to indicate that your findings do not provide direct evidence of replication in the blood, (as you have indicated in lines 330-334), and that replication of H5N1 in blood cells requires further exploration.

Discretionary Revisions:

1) In the abstract and introduction, I would suggest adding the term “avian” to make highly pathogenic influenza A virus become “highly pathogenic avian influenza. This will clarify the manuscript because the highly pathogenic designation refers to pathogenicity in chickens.

2) Line 74 of the introduction. I would suggest rewording this sentence. Influenza strains are numbered according to HA and NA subtype, and the terminology of HA and NA number is not generally used.

3) Line 138, the sentence begins with the number 6. This should be corrected to “Six”.

**Level of interest:** An article of importance in its field
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.