Author's response to reviews

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

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Author's response to reviews:

Dear Editor & Reviewer,

We have modified this manuscript and responded to your questions as noted below.

Editor: “The authors must delete the statement that highly pathogenic H5N1 viruses are readily transmissible from human to human. They are only rarely transmitted from human to human.”

A: We have deleted this statement and added: “There have been no reports thus far to indicate that highly pathogenic avian influenza H5N1 viruses are readily transmissible from human to human.”

Reviewer 2:

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.
A: We agree. We have revised representation of survival (%) of recipients in each group in Fig. 1B; changes in body weights in Fig. 2; changes in body temperature in Table 2, and corrected the appropriate sections in the text.

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%).

A: We agree and have corrected them in the text.

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.

A: We agree, we corrected to 1×103.6. We are unable to provide exact TCID50 conversions at this time and hope this is acceptable.

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.

A: We agree, we have deleted the word “successfully spread” to “occasionally transmitted”.

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.

A: We agree, we have made the changes in the text.

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.

A: We agree and have made the changes in the text.

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.

A: we agree, we deleted “The cell targets and host mechanisms that may support replication of highly pathogenic influenza virus in blood cells warrant further investigation.” from text and added “Although our current study does not provide direct evidence of replication in blood, H5N1 replication in blood cells may warrant 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”.

A: we agree, we corrected them in the text.