Author's response to reviews

Title: A case report of avian influenza H7N9 killing a young doctor in Shanghai, China

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Author's response to reviews: see over
Response letter
(Re: Manuscript: 1185759896151071)

Dear Neil and Editor-in-Chief:

Thank you very much for the letter. We are very pleased to receive the reviewers’ comments on our manuscript entitled “A case report of avian influenza H7N9 killing a young doctor in Shanghai, China” (Manuscript: 1185759896151071). We greatly appreciate these comments from the two reviewers who have provided to help us improve the quality of our paper. Therefore, we carefully revised the manuscript in accordance to both the reviewers’ comments and address the point-by-point response to each comment.

In order to help you know easier what and where we changed in the manuscript, we highlight the texts we changed in the file of “color version of the manuscript”. According to your suggestion, we asked a native English-speaking colleague to help us to revise the manuscript.

Yours faithfully,
Hao Pan

Note:
(1) Line is all referred to the manuscript version 2.
(2) We have highlighted the texts we changed in yellow.

I . Responses to the Comments from Reviewer #1

A. Major Compulsory Revisions

[Reviewer #1 Specific Comments]

1. Results on health care workers (HCW) screening are inconsistent. Is there any overlap between HCW blood samples tested by rRt-PCR and HAG?

[Answer]: Thanks for your good comments. You are right. Results on health care
workers (HCW) screening are inconsistent. All of throat swabs were negative but the two of 26 HCW were HA antibody positive. That is because all of throat swabs and serums were collected 9 days after the X onset. On one hand, the throat swabs were tested by rRT-PCR, which represented acute infection. The serums were tested by HAI, which represented the past infection. Y and Z were H7N9 positive in HA antibody because they have high dose and frequency to expose X patient.

In general, the results of two methods were inconsistent due to the different inter-period from the exposure to collection date, as well as the different exposure dose and cumulative hours in different HCW.

2. Ten HCW samples were screened for H7N9 by rRT-PCR. Indicate the collection date, line 140.
[Answer]: Both the blood samples and throat swabs, collected on January 20, 2014, were used to run for HAI and rRT-PCR tests, respectively.

We have improved it in the text highlighted in yellow.

b. Additional 35 close contacts, including 26 HCW were tested with HAG. Indicate the collection date, line 149.
[Answer]: We have improved it in the text highlighted in yellow.

c. It has to be emphasized, that there is no evidence that COPD-1 and SP-1 had avian influenza, since no tests were performed, line 155.
[Answer]: Thanks for your professional comments. There is no evidence that COPD-1 and SP-1 infected with avian influenza/H7N9. We emphasized this from two facts. All of the HCW caring for the two cases had no symptoms as well as 2 of 10 persons were H7N9 negative by HAI test. Therefore, we sincerely added one sentence at the end of last paragraph of discussion part. From the above results, there was no enough evidence indicating that COPD-1 and SP-1 had infected H7N9 avian influenza.

2. The serotiter (blood pair HAG test) of Y and Z doctors should have been performed at a later time point from a second blood sample in order to see, if there is an elevation in HAG serotiter, confirming the acute infection. Also, it would have been extremely important to get rRt-PCR results of their blood samples, since they were withdrawn 3 days after contact with Dr. X for doctor Z and 4 days after the X’s onset of illness in case of doctor Y, in order to detect possible active viremia. Blood samples were taken too early for serology, therefore the link between their seropositivity and exposure with X seems just a speculation. They (Z and Y) might have been exposed at an earlier time point from different source; therefore the conclusion on possible
human-to-human transmission is based on a very weak experimental data, line 227.

[Answer]: Thanks for your professional suggestion. Human-to-human transmission is based on the EPI link and viral features and experimental results. But really, the experimental data is very weak. So, this week we tested the blood samples of Y and Z collected 3 days after contact with Dr. X for doctor Z and 4 days after the X onset of illness, but both samples were negative in H7N9 RNA by RT-PCR. Your good comment will be helpful to improve the EPI investigation and design in the future, the double serum was necessary to confirm acute infection.

3. Important to mention when explaining phylogenetic relationships, that: HA, NA, PB2, NP virus genes from surgeon X are clustering together with an environmental sample A/environment/ShanghaiPD-JZ-012014, originating from LMP-B. Please define the exact source and GenBank Accession numbers, line 174. Also, this material was collected in current study and suggests a potential infectious source for X; therefore, it is unclear why authors excluded this possibility from their Discussion, lines: 219-222.

[Answer]: Thanks for your good suggestions. We have improved this discussion and added the GenBank. There was a potential possibility that X case was infected from the contaminated environment based on the evolution tree.

We added “HA, PA, PB2, and NP of JZ-1 are at the same branch as those of PD-2.” before the original sentence of “The PD-2 had more amino acid……” in line 185.

The original sentence “However, phylogenetic results did not indicate an LPM-oriented infection source (e.g. LPM-A, LPM-B) and this is consistent with epidemiological investigations that X had not visited LPMs” in line 227 was changed as “However, phylogenetic results did not indicate an LPM-A-oriented infection source, even X passed the entrance of LPM-A every day. Because four genes (HA, PA, PB2, NP) of JZ-1 clustered with PD-2, X’s infection might originate from LPM-B; but X wasn’t exposed directly to the environment and poultry from PLM-B. X’s mother in law might carry H7N9 virus to X’s home through the contaminated water in LPM-B.”

The original sentence “The two LPMs were an unlikely source” in line 231 was changed as “The LPM-A was an unlikely source”.

4. Avian influenza virus from patient X was deposited in the Genbank and compared to influenza viruses from two live poultry markets and other human cases from the past. Were these cases lethal as well, what are they GenBank Accession numbers?

Lines: 174, 178.
PD-2, JZ-1, and CN-2 were lethal. We have Genbank numbers of them. We added “GenBank accession numbers of PD-2, JZ-1, and CN-2 are as follows: PB2 (KJ549799, KJ549791, KJ549783), PB1 (KJ195791, KJ549792, KJ549784), PA (KJ549800, KJ549793, KJ549785), HA (KJ195792, KJ549794, KJ549786), NP (KJ195793, KJ549795, KJ549787), NA (KJ195794, KJ549796, KJ549788), M (KJ195795, KJ549797, KJ549789), NS (KJ195796, KJ549798, KJ549790)” at the end of line 181.

B. Minor revisions:

1. Provide the reference for rRt-PCR, line 119.
   [Answer]: We ran all rRT-PCR tests according to the SOP from World Health Organization. “World Health Organization: Real-time RT-PCR Protocol for the Detection of Avian Influenza A (H7N9) Virus. http://www.who.int/influenza/gisrs_laboratory/cnic_realtime_rt_pcr_protocol_a_h7n9.pdf?ua=1” was a new reference and added before line 288.

2. The 16 environmental samples collected from ICU, what are they exactly? Line 158.
   [Answer]: Thanks for your comments. We have added 16 environmental samples in detail in the text.

3. The abbreviations have to be consistent, like SH-PDH and PDH indicates the same thing, lines: 87, 96. LMP, HCW has to be explained in the abstract, lines: 66 and 60. Unnecessary abbreviations, if they are not repeating in the text one more time, has to be removed (example U-ER, line: 123), as they make the text congested and hard to read.
   [Answer]:
   (1) The original sentence “poultry nor had he visited live-poultry markets” in line 58 was changed as “poultry nor had he visited live-poultry markets (LPMs)”.
   (2) The original sentence “close contacts identified two HCWs” in line 60 was changed as “close contacts identified two healthcare workers (HCWs)”.
   (3) The original words “admission into PDH” in line 96 was changed as “admission into SH-PDH”.
   (4) The original words “U-shaped emergency room (U-ER)” in line 123 was changed as “U-shaped emergency room”.
4. For clarity, PPE (Personal Protective Equipment), has to be used all other the text instead of personal protective measures (PPMs).

[Answer]:
(1) The original words “any personal protective measures (PPMs)” in line 142 was changed as “any personal protective equipment (PPE)”.
(2) The original word “PPMs” in line 228 and line 238 was changed as “PPE”.
(3) The original words “PPM: personal protective measure” in line 357 was changed as “PPE: personal protective equipment”.

5. It needs noting, that prophylactic (per-exposure ) treatment of health care workers with oseltamivir or other available drugs, during epidemics, provides better protection than post exposure measures., line 236.

[Answer]: You are right. We agree with you. We improved this sentence.
The original sentence “(1) elevating the alertness of HCWs to the need for early antiviral treatment once they develop ILI-symptoms” in line 236 was changed as “During epidemics of H7N9 influenza, those HCWs who might be exposed to H7N9 cases are advised to take oseltamivir or other available drugs against H7N9 viruses in advance.”

Responses to the Comments from Reviewer #2

Minor Essential Revisions:

1. In the "Abstract", treatment for the patient should be included in the "Case presentation" because the authors mentioned "...late treatment..." in "Conclusions".
[Answer]: Based on your comments. We put the treatment for this patient in "Case presentation". Thanks.

2. It will be much clearly for readers if the authors can make a list of all the abbreviation they used in the manuscript, since there were so many abbreviations which bring confusion and misunderstanding.
[Answer]: All abbreviations were added after the part of text.

3. Figure 2 legend needs more detail information, including wording and arrow in the figures.

[Answer]: The legend of figure 2 has been revised. We also added arrows in the figure 2.

The original sentence “Figure 2 Chest Radiograph of case His chest radiograph was taken on January 17, 2014 (i.e. day 6 after onset of illness)” was changed as “Figure 2 Representative radiographic findings of the laboratory-confirmed 31-year-old Shanghai surgeon infected with H7N9 influenza. Chest radiograph of this patient was taken at 7 days after onset of symptoms, showing bilateral pulmonary infiltrates of airspace consolidation and severe consolidation in the left lobe.”
4. Page 3, Clinical history, giving detailed temperature of the fever will be better.

[Answer]: Thanks for your professional suggestions.

The patient’s temperature was taken only once from onset of his illness to death. The patient was a doctor and he felt he had a fever when he became ill on January 11th until admission to the hospital but he didn’t have his temperature taken. On January 15th, the patient sought medical service at the hospital where he worked and knew the physicians quite well in the outpatient department. Therefore, he didn’t have his temperature taken either. His temperature was only taken on January 17th, which was 39 degrees Celsius, but he died next morning.

Comments from the authors

1. The original sentence “the first healthcare worker (HCW) succumbed” in line 80 was changed as “the first HCW succumbed”.

2. The original sentence “The two LPMs were an unlikely source of his infection, but a contaminated environment” in line 66 was changed as “The LPM he passed every day was an unlikely source of his infection”.

3. The original sentence “passed by the live-poultry market (LPM)” in line 112 was changed as “passed by LPM”.

4. The original sentence “Specimens from 12 persons [COPD-136 1, SP-1, 10 HCWs] were not available for H7N9 tests. Follow-up of 10 HCWs who” in line 136 was changed as “Specimens from 10 persons [COPD-1, SP-1, 8 HCWs except Dr.-A1 and Dr.-B1] were not available for H7N9 tests. Follow-up of all 10 HCWs who”.

5. The original words “LPMs (14.63% and 18.52%)” in line 216 was changed as “LPMs (14.6% and 18.5%)”.

6. The original sentence “The work was supported by grants from the Shanghai Municipal Commission of Health & Family Planning (2013QLG001) and the Ministry of Science & Technology of China (KJYJ-2013-01-01-01 and KJYJ-2013-01-05).” in line 270 was changed as “The work was supported by grants from the Shanghai Municipal Commission of Health & Family Planning: Key Discipline: Epidemiology (No. 12GWZX0101) and 2013QLG001 and the Ministry of
7. **Number of References.** Line 288: 5 was changed to 6. Line 292: 6 was changed to 7. Line 295: 7 was changed to 8. Line 298: 8 was changed to 9. Line 300: 9 was changed to 10. Line 304: 10 was changed to 11. Line 307: 11 was changed to 12. Line 309: 12 was changed to 13. Line 315: 13 was changed to 14. Line 319: 14 was changed to 15. Line 322: 15 was changed to 16. Line 327: 16 was changed to 17. Line 332: 17 was changed to 18. Line 336: 18 was changed to 19. Line 339: 19 was changed to 20. Lined 342: 20 was changed to 21.

8. The original words “A/Chicken/Shanghai/PD-CN-2/2014” in line 174 and Line 385 was changed to “A/Chicken/Shanghai/PD-CN-02/2014”.

9. The original words “Identity with SH/PD-02/2014” in line 348 in table 2 was changed to “Identity with PD-2”.

10. The original sentence “SH/PD-2/2014: PD-2, the surgeon’s H7N9 isolate; PD-2: another human case” in line 349 was changed as “PD-2: the surgeon’s H7N9 isolate; PD-1: another human case”.

11. The original sentence “Viruses from the two PD cases (PD-1 and PD-2) belonged to the same sub-lineage in HA, NA, M and NS genes whereas the two LPM-Env-H7N9 isolates belonged to another sub-lineage.” in the legend of figure 4 was deleted.