Author's response to reviews

Title: Interspecies interactions and Influenza A virus risk in small swine farms in Peru

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Author's response to reviews: see over
First and foremost, many thanks to the reviewers for their helpful comments and suggestions. We have worked to address all of them in our revised manuscript. Our responses and a description of the changes that we have made are provided below.

Reviewer Javier J Guitian

Reviewer’s report:
The manuscript presents findings that are novel and relevant for the control of influenza infection and to some extent also for other animal diseases / zoonoses. The methods are sound and well described, results clearly presented and conclusions justified. I have however a series of specific points to be considered which are listed below and more general point.

Major compulsory revisions: none
Minor essential revisions: 1, 2, 5, 6, 8, 9, 14, 16
Discretionary revisions: 3, 4, 7, 10, 11, 12, 13, 15, 17

1) The more general comment I want to make relates to the structure of the information provided. In the results and discussion section it is not always clear whether specific information provided is relevant for poultry to swine transmission, poultry to humans transmission, swine to humans transmission or humans to swine. It would greatly help the readers if a framework clarifying the different transmission pathways being studied in this paper was presented in the methods section and this framework was then used to present the results and to discuss them. Some practices may be relevant to one of the above interspecies transmission routes and not to others, by introducing a certain structure in the methods that is later used in results and discussion the findings will be more useful for the readers.

Many thanks for this excellent recommendation. We have now established the framework for the different types of interactions in the methods section and have followed the same order/structure in the results and discussion sections. We have tried to make this explicit by presenting the pathways as follows in each of the three sections: avian-swine interactions; human-avian interactions; human-swine interactions; and hygiene practices.

2) Abstract: the methods section includes some results – the focus should be on the methods, which, even if they are qualitative should be described (observers, timing and method of observations etc.).

This was our oversight. We have now included only methods in the methods section and moved the results to the results section.

3) Background: “areas where industrialized farming is infrequent” why not simply saying areas where small scale intensive production is frequent (and important for local livelihoods).
Many thanks. We have made this change.

4) Methods: Study Participants. The first paragraph (“Because this... no longer emerged”). Should be expanded and clarified: the authors explain the rationale for the lack of consideration of a pre-defined sample size; they should also justify / explain to the readers purposive selection (as opposed to probabilistic) – I am not questioning the appropriateness of purposive selection, I am just asking for this to be made clearer to the readers.

Your comment helped us to see that our explanation of purposive sampling was unclear. Thank you. We have now lengthened this section to clarify that we used purposive/maximum variation sampling. We have also explained how we did so step by step. The text is as follows:

Because this is a qualitative study, a sample size calculation was not done. Instead, we used purposive sampling to literally seek out participants “with a purpose,” that of being able to provide in-depth information about swine-associated interspecies interactions. We employed maximum variation purposive sampling, which aims to select participants who represent a broad range of possible variations in the topic of interest [26]. For this reason, we selected the two settings just described, confined pig farming in Chancay and low-investment pig farming in Tumbes. Additionally, within each setting, we sampled from a range of farms that varied according to the criteria that are most relevant to the realities of pig farming and possible interspecies interactions in the two contexts (Table 1). In Chancay, since all of the farms are located at different positions on the same hills above the ocean, we sampled from farms that are situated at different heights on those hills. In Tumbes, there are farms in all of the different villages, which are located at different distances from Tumbes. Therefore, we sampled from farms in 17 villages, which were classified as short, medium and far distance to Tumbes city. We also sampled from those that were close to the coast, with presence of wild birds, given that wild birds in Peru have been found to be reservoirs of IAVs. We sampled participants until reaching a point of saturation, when new data no longer emerged.

5) In relation to the same issue (selection of participants), were the criteria used in Chancay and Tumbes comparable? Did the fact that the work in Tumbes was integrated within ongoing research activities result in different selection criteria?

Please make this clear to the readers.

This is an excellent question. The criteria that we used were based on the reality of pig farming in each study location. For example, all of the pig farms in Chancay are located in the same area, in different locations on the hills. Therefore, visiting all of the pig farms was quite “easy” in terms of logistics since they are within walking distance of one another. In Tumbes, the pig farms are distributed across different villages, which can be reached only by vehicle. Therefore, we benefited from ongoing research activities in Tumbes in that the research team had contacts in these different villages and we were therefore able to visit many of them.

6) Methods: Data collection activities: Direct observation: who were the interviewers? It is said that “the researches” but more information is needed: how many researchers? Always the same team? If not, was there some type of harmonization phase of the process? – the discussion suggests that there was an element of standardization of the process, please explain this stage of the
research in the methods.
Again, an excellent question. We have now integrated additional information about who carried out the observations and interviews. Also, we have described the harmonization/standardization phase for the direct observation method. The text is as follows:

Observations were carried out by two of the authors (SM and MAR). Both observers were trained by AMB and then carried out observations of two of the same pig farms in Chancay in order to apply the lessons learned during training. Following these observations, SM, MAR and AMB met to review the two sets of observations line by line, discuss any differences, and establish guidelines to standardize observation practices.

7) To facilitate the readers’ understanding of the work and maximize transparency I suggest the interview guides are made available either electronically as supplementary material or available upon request to the authors (this is just a suggestion).
Yes, we would be happy to provide the interview guides upon request.

8) Methods: Data Analysis: this section should be slightly expanded so that it is clearer to the readers which associations have been tested. No need to list specific 2-way associations but at least they should be broadly indicated.
We have now specified that the difference between groups refers to confined farming in Chancay versus low-investment farming in Tumbes.

9) Methods: what constitutes an “interaction” has to be precisely defined in the methods, so that authors know exactly how to interpret table 2.
We have now defined the different types of “interactions” in the Methods section, under Data collection activities, Direct observation.

10) Results: Hygiene practices: boots “are considered to be protective against disease transmission”, please clarify, transmission from ? to?. Protective if they are disinfected etc. I guess.
We have removed this since it was unclear.

11) Discussion: the authors highlight sample size as a limitation of this, but this somehow contradicts what is stated in the methods – that size was determined by saturation i.e. no additional useful data being obtained. I am personally not that worried about sample size in this study...
We agree that sample size is not necessarily a limitation. However, we think that many more quantitatively oriented readers may consider it to be a limitation and therefore think that it is important to continue to include this information.

12) Discussion: are there any data on influenza infection in pigs in Peru available? If so please refer to this in the discussion.
Our study group is currently collecting this data but results are not yet available.

13) Discussion: study findings may also be relevant to transmission of other diseases, please refer to this, if possible giving some examples of relevant poultry/swine diseases/zoonoses prevalent in Peru.
We have now included this information at the end of the discussion section.
Reviewer’s report:
Review of Interspecies Interactions and Influenza A virus risk in small swine farms in Peru by Sarah McCune et al.
In this qualitative study, McCune and colleagues investigated interspecies interactions among human, swine, and avian species in small-scale swine farms located in Peru. Because certain genotypes or subtypes of influenza A are able to transmit among these species through an occasional direct-contact, monitoring of interspecies interactions is very important for public health. In this study, based on the size and raising systems, the swine farms tested likely shared similarities with those in other developing Asian and African countries where H5N1 HPAI infections of humans occurred frequently. Thus, the findings might be extrapolated to estimate interspecies interactions in other similar regions, such as Asia or Africa. As risk factors of interspecies transmission of influenza virus in swine farms of Peru, the authors suggested: 1) high human-swine interactions, 2) unexpected contact of swine with avian species via feeding poultry mortality, 3) suboptimal hygiene and biosecurity practices in swine or poultry farms, requiring the related, solid guidelines or education for farmers. This manuscript is very well-written as supported by excellent discussion and two impressive photos, but with minor corrections or suggestions by this reviewer as follows:

Abstract
1. The result sentences, “In both locations, ~ at both locations”, need to be removed from the Methods section.
Thank you. We have removed the results sentences for the methods section.

2. After the first sentence in Conclusions, this reviewer suggests to input a sentence as follows: “Virological and serological surveillance for influenza viruses will also be required in these human and animal populations.”
Thank you for this excellent suggestion. We have included this sentence in the Abstract and in the main text.
Background
1. In the 2nd paragraph, the fact that avian influenza viruses are able to transmit and infect a variety of mammals (companion pets or livestock) as the following examples: 1) dogs by avian H3N2, 2) cats by avian H5N1, and 3) pigs by avian H5N2, could be addressed.
   We have now included this information in the Background section. The text reads: Evidence from Asia shows that avian influenza viruses are also able to transmit to and infect a variety of mammals. There are several examples of the infection of dogs by H3N2 [8-10], an example from Cambodia of the probable infection of five cat species by H5N1 [11], and isolation of the H5N2 virus in pigs in Korea [12].

2. The 1st sentence of the 3rd paragraph can be revised as follows:
   “Interspecies transmission can occur by jumping or adaptation of a whole virus to a new host species, or through the process of reassortment-”
   Thank you. We have made this change.

Methods
1. In the 2nd paragraph of the Study participants section, please provide the background or some information on each individual composing of the research team, for example, which scientific backgrounds they have (in virology, epidemiology, or veterinary science etc.)?
   We are unsure about whether it is appropriate to include this information in the manuscript. We are including it here and will defer to the Editors as to whether or not it should be incorporated into the manuscript.
   SM, MAR and AMB all have training in social sciences and AMB has a PhD in public health. CSA is a veterinarian with PhD-level training in public health. RHG is an MD, DTMH. VA is a veterinarian. VAC and AEG are veterinarians with PhDs in public health. JMM has a PhD in biology.

Discussion
1. In the 1st paragraph, the authors could simply introduce an interesting, similar case in a previous report (Song et al., Emerging Infectious Disease. 2008), addressing that dead poultry fed dogs in South Korea were infected by avian H3N2 influenza virus, and poultry mortality is a significant cause of interspecies transmission of avian H3N2 to dogs.
   We have now included this information. Since we re-ordered the discussion section, it is located in paragraph 4:
   It is important to note here that dogs in South Korea were infected by avian H3N2 influenza virus, most likely from being fed infected poultry byproducts [30].