nature portfolio

Peer Review File

Zoonotic transmission of novel Influenza A variant viruses detected in Brazil during 2020 to 2023

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This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
Authors present eight cases of H1v and H3v human infection in Brazil between 2020 and 2023. It presents that the human cases are in three major lineages 1A.3.3.2 (pdm09), 1B (human-like), and H3 1990.5 as well as their epi information about how and where to collect the samples. The information provided is well described to understand the current situation of variant virus circulating in Brazil. However, there are some considerations to improve the manuscript.

The description of genetic markers in the manuscript is not enough to understand the human cases of Brazil to the readers. Authors need to present the genetic markers including mutations found in detail how impact their mutations on the virus transmissibility, antiviral resistance, or virulence.

The number of mutations (47-127) seems very high compared to the current Southern Hemisphere vaccine strains. Authors alternately need to compare the sequences to other available vaccine candidate.

Reviewer #2

(Remarks to the Author)
The manuscript by Resende, Junqueira, Tochetto, and collaborators describes the epidemiological investigation of putative introductions of variant Influenza A viruses from swine to humans in Brazil. These findings are important as they increase the body of literature on enzootic transmissions from pigs to humans, as well as back spill of human influenza viruses previously introduced to swine. The manuscript has a detailed description of the surveillance programs in Brazil deployed for the detection of both human and swine influenza viruses by the corresponding agencies. Despite these programs the findings highlight the challenges to identify and investigate these transmissions in a timely manner.

Overall the manuscript is well written and the methods well employed providing a comprehensive phylogenetic characterization to determine the potential origin of the sequenced viruses. Of interest are the findings for the cases in Toledo, although less than a handful were identified they span a three year period. The phylogenetic clustering of these viruses across all gene segments suggest that they might have originated from a single introduction from swine, with potential onward transmission in humans for at least these three years. The authors are rightly cautious about over-interpreting their findings, due to a lack of swine sequences from the same period (2021-2023), however, the time period that encompasses these 4 cases should be clearly mentioned in the text. One would expect that the likelihood of independent introductions to cluster together over a three year period would be low. The findings certainly warrant further follow up to rule out human-to-human transmission of these viruses. Additionally, a time-scaled analysis or a concatenated phylogeny (i.e. all segments treated as one long genome) could also help determine if these viruses share a common ancestor and help estimate a time of introduction relative to the latest swine viruses, but I understand that these inferences would be limited by the number of swine sequences available.

A few remarks on the text and figures (line numbers would've been appreciated):

Introduction: In my opinion the introduction could be used to describe the surveillance system in Brazil for humans, while the general description of Influenza A viruses and classifications of the swine genotypes could be shortened. The human

surveillance system is described in the methods but referencing several other publications, which is why I think it would better fit the introduction, where the swine surveillance by EMBRAPA is also described.

Abstract: Spell MRT-PCR as it is not standard nomenclature. Multi-segment or Multiplex?

Introduction, second paragraph: add a hyphen to “a2.3” for consistency with a-2.6.

Figures: For the phylogenies containing more than one case it was hard to locate each case in relation to the description in the text. In the text the cases are referred by case number, whereas in the tree they appear by strain name. Please use different colors for each case to depict them in the tree and make them easier to find. This is important to make it easier to visualize and interpret those that cluster together, and also when looking at other gene segments in the supplementary figures.
Figure S3 is missing the case designations.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
Authors present the genetic markers and their description on each virus from Brazil. This information is beneficial to the reader for understanding the genetic situations of the recent circulating viruses in Brazil.
Also, the amino acid difference between the viruses from Brazil and other CVV is well provided. The additional features make the strong support for the founding in the manuscript.

Reviewer #2

(Remarks to the Author)
The authors have addressed my comments satisfactorily. While Table 2 is sufficient in this case to show that the H1v and H3v show are indeed separate introductions, I will argue that the phylogenies with concatenated genomes would effectively show how reassortment separates (or clusters) these introductions from a single common ancestor. For example, concatenated phylogenies have been used recently to show the origin and spread of the avian Highly Pathogenic H5N1 introductions to cattle and other host species by multiple groups (https://www.biorxiv.org/content/10.1101/2024.05.01.591751v1.full#F2, https://nextstrain.org/blog/2024-06-18-h5n1-cattle-outbreak-analysis-and-resources, https://academic.oup.com/ve/article/10/1/veae031/7645834, https://virological.org/t/preliminary-report-on-genomic-epidemiology-of-the-2024-h5n1-influenza-a-virus-outbreak-in-u-s-cattle-part-1-of-2/970).

REVIEWER COMMENTS

We would like to thank the reviewers for their critical and insightful review of our manuscript. We have followed the reviewer's suggestions and modified the manuscript in line with their comments. We sincerely feel that the reviewer's comments have significantly improved our manuscript.

Some adjustments were performed, such as: Abstract was reduced to 150 words and others according to the GUIDE TO FORMATTING ARTICLES.

Below are our point-by-point responses to the reviewer's comments.

Reviewer #1 (Remarks to the Author):

Authors present eight cases of H1v and H3v human infection in Brazil between 2020 and 2023. It presents that the human cases are in three major lineages 1A.3.3.2 (pdm09), 1B (human-like), and H3 1990.5 as well as their epi information about how and where to collect the samples. The information provided is well described to understand the current situation of variant virus circulating in Brazil. However, there are some considerations to improve the manuscript.

The description of genetic markers in the manuscript is not enough to understand the human cases of Brazil to the readers. Authors need to present the genetic markers including mutations found in detail how impact their mutations on the virus transmissibility, antiviral resistance, or virulence.

Answer: To better describe the genetic markers we include a supplementary table (Table S3. Features of previously reported genetic markers associated with antiviral resistance, virulence and host specificity shift.). This table shows details of mutation found in the gene segments and proteins of Influenza A variant viruses. In the manuscript, we can observe a better description of this topic in lines 362-381 (Results) and lines 444-447 (Discussion).

The number of mutations (47-127) seems very high compared to the current Southern Hemisphere vaccine strains. Authors alternately need to compare the sequences to other available vaccine candidate.

Answer: *Two supplementary figures (Suppl. Figure 7 and 8) were created based on this suggestion, showing the CVVs and vaccine strains recommended from 2020 to 2024.*
https://www.who.int/teams/global-influenza-programme/vaccines/who-recommendations/zoonotic-influenza-viruses-and-candidate-vaccine-viruses
There is no specific CVV to the swine clades related to the variants found in Brazil. Unfortunately, neither we nor the CDC in Atlanta could isolate the Influenza A variant viruses. EMBRAPA was also contacted to verify if they had a positive swine clinical sample or isolate close to the human variant cases described recently in Brazil. Such a sample or isolate was not found at Embrapa's collection.

Reviewer #2 (Remarks to the Author):

The manuscript by Resende, Junqueira, Tochetto, and collaborators describes the epidemiological investigation of putative introductions of variant Influenza A viruses from swine to humans in Brazil. These findings are important as they increase the body of literature on enzootic transmissions from pigs to humans, as well as back spill of human influenza viruses previously introduced to swine. The manuscript has a detailed description of the surveillance programs in Brazil deployed for the detection of both human and swine influenza viruses by the corresponding agencies. Despite these programs the findings highlight the challenges to identify and investigate these transmissions in a timely manner.

Overall the manuscript is well written and the methods well employed providing a comprehensive phylogenetic characterization to determine the potential origin of the sequenced viruses. Of interest are the findings for the cases in Toledo, although less than a handful were identified they span a three year period. The phylogenetic clustering of these viruses across all gene segments suggest that they might have originated from a single introduction from swine, with potential onward transmission in humans for at least these three years. The authors are rightly cautious about over-interpreting their findings, due to a lack of swine sequences from the same period (2021-2023), however, the time period that encompasses these 4 cases should be clearly mentioned in the text. One would expect that the likelihood of independent introductions to cluster together over a three year period would be low. The findings certainly warrant further follow up to rule out human-to-human transmission of these viruses. Additionally, a time-scaled analysis or a concatenated phylogeny (i.e. all segments treated as one long genome) could also help determine if these viruses share a common ancestor and help estimate a time of introduction relative to the latest swine viruses, but I understand that these inferences would be limited by the number of swine sequences available.

Answer: The time-scaled analysis of swine clades has already been estimated by Tocheto et al. (Viruses, doi: 10.3390/v15020576) and Junqueira et al. (Front. Microbiol. 10.3389/fmicb.2023.1243567). The time-scaled of the most recent common ancestor of the variants isolated in humans represents a refinement in the collection date of the swine sequences. Unfortunately, we do not have this information uniformly. The detection of these variants in humans was sporadic, with a well-defined epidemiological link, and there were no reports of continued transmission among the contacts. The onset of symptoms marks the introduction of these variant viruses into humans.
The analysis of concatenated genomes is not common for influenza viruses due to the different origins of each segment. We know that the variants found are distinct viruses that have undergone reassortment. The alignment of these concatenated genomes would not be possible due to these different origins. Table 2 shows the reassortment between the segments.

A few remarks on the text and figures (line numbers would've been appreciated):
Answer: Line numbers were added in the manuscript file.

Introduction: In my opinion the introduction could be used to describe the surveillance system in Brazil for humans, while the general description of Influenza A viruses and classifications of the swine genotypes could be shortened. The human surveillance system is described in the methods but referencing several other publications, which is why I think it would better fit the introduction, where the swine surveillance by EMBRAPA is also described.

Answer: We agreed and the description of the surveillance system in Brazil for humans was moved to the introduction (l. 116-124).

Abstract: Spell MRT-PCR as it is not standard nomenclature. Multi-segment or Multiplex?
Answer: It was corrected in the text, line 195 methods. It means multi-segment-RT-PCR (M-RT-PCR), such as described in the original paper (Multisegment reverse transcription-PCR (M-RT-PCR) is used by DOI: 10.1128/JVI.01109-09 and in the review paper DOI: 10.1007/978-1-61779-621-0_11).

Introduction, second paragraph: add a hyphen to "a2.3" for consistency with a-2.6.
Answer: It was corrected.

Figures: For the phylogenies containing more than one case it was hard to locate each case in relation to the description in the text. In the text the cases are referred by case number, whereas in the tree they appear by strain name. Please use different colors for each case to depict them in the tree and make them easier to find. This is important to make it easier to visualize and interpret those that cluster together, and also when looking at other gene segments in the supplementary figures.
Figure S3 is missing the case designations.
Answer: As suggested, we created a colour scheme for discrimination of the cases along the phylogenetic trees. Case designation was included in Figure S3.