Cover Letter

Manuscript ID: IDOP-D-24-00461

"Molecular characterisation of human rabies in Tanzania and Kenya: a case series report and phylogenetic investigation"

Dear Editor,

On behalf of my co-authors, we extend our gratitude to the reviewers for their valuable feedback on the manuscript.

We've incorporated their comments and provided detailed explanations below, referencing the corresponding line numbers in the revised manuscript. Additionally, we have revised the supplementary files, including tables and figures. We do hope that with our revisions this manuscript will be acceptable for publication in the journal *Infectious Diseases of Poverty*.

Sincerely,

Gurdeep Jaswant

University of Nairobi

On behalf of co-authors

Reviewer reports:

**Reviewer #1: General comments**

The manuscript is well-written and contributes significantly to the understanding of rabies in East Africa. It effectively combines epidemiological and molecular data to provide a comprehensive analysis. Minor grammatical and typographical errors need to be addressed.

A thorough proofreading would enhance the overall quality of the manuscript.

1. Title: The title is clear and concise, accurately reflecting the content of the manuscript. However, it would be beneficial to specify the countries involved in the study for greater specificity.

*Thank you for this feedback. We have added the countries involved in the study to the title as per your suggestion: “Molecular characterisation of human rabies in Tanzania and Kenya: a case series report and phylogenetic investigation.” (lines 1-2)*

1. Abstract: The abstract is well-structured, summarising the background, case presentation, results, and conclusion effectively. It might be helpful to briefly mention the main findings related to the phylogenetic investigation in the abstract.

*We have elaborated the results from the phylogenetic investigation (“The phylogenetic investigation highlights the transboundary circulation of rabies within domestic dog populations, revealing distinct rabies virus clades with evidence of regional spread”, lines 49-50. We have also added an extra line interpreting how these results should inform rabies control “These findings underscore the importance of coordinated cross-border control efforts between the two countries.'' (lines 49-52)*

1. Introduction: The introduction provides a comprehensive overview of the rabies situation in East Africa. However, it could benefit from a more detailed discussion on the global context of rabies and why East Africa is a critical region for this research.

*We have edited the introductory paragraph to explain why East Africa is a region of interest for this research, particularly given Gavi-eligibility for support to improve access to human rabies vaccines (lines 65-71).*

1. Consider including more recent statistics and references to provide an updated context.

*We now cite a more recent study that used new empirical data from both countries to revise country specific estimates of human rabies deaths (WHO rabies modelling consortium 2019).*

1. Case Presentation: The case presentations are detailed and provide valuable insights into each case. The narrative style is engaging, but some sections could be more concise. It would be helpful to include a table summarising the key details of each case for easier reference and comparison.

*Thank you. We have updated Table 1 to better summarise details of each case based on the feedback from reviewers and the editor.*

1. Methodology: The methodology section is thorough, detailing the processes of data collection, laboratory analysis, and phylogenetic investigation. However, some parts are overly technical and could be simplified for a broader audience.

*Thank you. We have tried to simplify the language wherever possible.*

1. Clarify the selection criteria for the cases included in the study to ensure transparency.

*We have added the sentence “There were no specific selection criteria for choosing the samples included in this study. Rather, the samples analysed were available due to an ongoing surveillance project in these two areas, which provided the necessary dialogue with affected families to enable sample collection and to undertake follow up.” (lines 96-99)*

1. Results: The results are presented clearly, highlighting the epidemiological context and the findings from the genomic data. The discussion on the transboundary circulation of rabies is particularly insightful. Consider providing more visual aids, such as phylogenetic trees or maps, to enhance the presentation of the results.

*We have tried to better emphasise how transboundary transmission is shown by the maps and phylogenies in figures 2 and 3, that are coloured by lineage to illustrate the cross-border circulation.*

1. Discussion: The discussion effectively connects the findings to broader public health implications. The emphasis on the need for improved awareness and accessibility of post-exposure prophylaxis (PEP) is well-placed. Expand the discussion on potential barriers to PEP administration and how these might be addressed, drawing on examples from other regions or diseases.

*We have added a paragraph on barriers to PEP access and potential to overcome these “Potential barriers to accessing PEP include lack of awareness among healthcare providers and the public, and economic decisions that prevent the vaccine being stocked or made available free-of-charge to patients. Many healthcare facilities in endemic regions are under-resourced and healthcare practitioners may not be adequately trained to administer PEP correctly, leading to inconsistent practices and poor patient outcomes. To address these barriers, there is an urgent need to update national guidelines to follow WHO guidance and ensure healthcare practitioners are trained and competent to manage rabies exposures appropriately. WHO has developed new policies and guidance for countries [1,31], and Gavi’s support for human rabies vaccines provides an opportunity to update national guidelines, and operationalize these through vaccine procurement and distribution, with delivery of training packages for health care practitioners and integration of guidance into health curricula. Community education campaigns are also crucial to raise awareness about the importance of timely PEP and proper wound care after an exposure.” (lines 353-364)*

1. Conclusion: The conclusion is strong, summarising the main points succinctly and providing clear recommendations. It could be enhanced by highlighting the next steps for research and policy based on the findings. Emphasise the importance of coordinated dog vaccination programs as a long-term solution to rabies control.

*Based on an editorial request we have moved key findings and their policy implications to the first paragraph of the discussion and conclusion section. In the final paragraph we now emphasise the importance of scaling up and sustaining dog vaccination to interrupt rabies transmission and mitigate against transboundary circulation, as well as highlighting how continued genomic surveillance can be used to evaluate these control strategies: “As countries pursue the global ‘Zero by 30’ goal to eliminate human deaths from dog-mediated rabies [38], cross-border dog vaccination programs must be emphasised as a long-term solution to rabies control. Genomic approaches have the potential to enhance rabies surveillance and provide actionable information, for example, by revealing transboundary spread. We urge regional coordinated action towards this goal to prevent these tragic deaths and recommend that future research and policy focus on comprehensive dog vaccination to control rabies and enhanced surveillance to evaluate progress”. (Lines 389-395)*

1. Specific Comments:
2. Page 3, Line 20: Clarify the definition of "high-income countries" as it pertains to rabies elimination strategies.

*We have removed the clause “high-income” and rewritten the sentence to just explain that in East Africa dog rabies vaccination has not been carried out at scale in contrast to countries that have eliminated rabies through dog vaccination. We have also added a sentence highlighting how this is particularly detrimental given the limited access to post-exposure vaccines: “Moreover, economic constraints, lack of awareness, and logistical challenges hinder access to post-exposure vaccines which are essential to prevent rabies deaths when the disease still circulates in domestic dog populations [7].” (lines 65-68)*

1. Page 5, Line 45: Consider rephrasing for clarity: "The rabies virus is highly neurotropic..."

*We have rephrased the sentence to “The rabies virus targets the nervous system, travelling along nerves from the site of infection to the brain, where it multiplies and causes rabies.” (lines 72-73).*

1. Page 7, Table 1: Add a column for the timeline of events (bite, onset of symptoms, healthcare visits).

*Thank you for the comment, the timeline of events are now in the table and structured to match the points suggested: “bite = Exposure date, onset of symptoms = Incubation period till symptom onset, and healthcare visits = Delay to attend health facility after exposure.*

1. Page 9, Line 10: Provide more detail on the off-label intramuscular regimen mentioned.

*We now explain that an off-label intramuscular regimen means administering the vaccine in a way that is not aligned with WHO recommendations (line 114), as clarified in the discussion (lines 344-352).*

**Reviewer #2:** The article is good as it is. I don't have any major changes to suggest.

*Thank you - we are delighted by your feedback!*

1. I recommend you to check all the references because some of them (6, 26) are not correct. I didn't do all of them but I found one that needs revision.

*Thank you - we have now checked these thoroughly and corrected those with mistakes.*

1. I suggest that the references in the first paragraph, which are more than 10 years old, are replaced by more recent ones, especially those that deal with the number of rabies cases in the world. Are 59,000 people still dying per year? Has this number not declined in the last decade?

*We appreciate that the 2015 reference is now old. A lot of data was compiled and reviewed for the WHO rabies modelling consortium estimates to advocate for Gavi’s investment in human rabies vaccines, so we now refer to the country estimates reported there.*

1. Regarding the figures, I think their quality could be better, since when we increase the size, to see some detail, they lose resolution and become unreadable.

*Sorry, we think this was a mistake in the uploaded figures, because the original are high quality vector images. We have made sure that the uploaded revisions use the high quality figures.*

**Editor's comments:**

1. Graphical abstract image:

1. The graphical abstract image should with a resolution of at least 300 dpi (dots per inch), and the image size is approximately 920 (width)\*300 (length) pixels.
2. Do not use the map. This is a picture which will appear underneath the Abstract in the article PDF. You could find an image which is relevant to the topic covered and serve to attract readers' attention to the article.

*We have submitted a revised graphical abstract that omits the map that is included in the figures and instead uses a map schematic to illustrate our finding of cross-border circulation.*

2. Authors and author affiliations:

1. Author name should be: Given/First name+ Family/Last name. Check that each author name is spelled correctly, and that names appear in the correct order of first name followed by family name. Please check the highlighted name.

*We have carefully reviewed the names, and confirm that all the authors' names are spelt correctly and presented in the correct order.*

1. The corresponding author is usually placed at the last. Please consider placing the authors with equal contributions together at the beginning.

*We have changed the corresponding author and indicated a sole senior author in line with the journal guidance. But we also wonder if this authorship guidance is outdated? Our impression is that increasingly the contributions of authors are presented more equitably (in our case, the 3 senior authors all supervised the student, and the lead author is liaising with the journal!).*

1. The sequence of author affiliation numbers should be arranged in the order they appear. Affiliation number 16 appears before numbers 14 and 15.

*Thank you, we have corrected this error.*

1. Please write the authors' affiliations in the following unified format: Department Name, Institution Name, City Postal Code, Country.

*We have now followed this format.*

3. Abstract and Keywords:

1. The first persons (such as I, we, us, our) are not advised in abstracts.

*We have revised the abstract to not use the first person..*

1. Background: Please condense this section into two sentences, the first sentence introducing the research background or necessity, and the second sentence explaining the purpose of the research.

*We have condensed this section into two sentences “Rabies remains a major public health problem in low- and middle-income countries, with human rabies deaths rarely being laboratory-confirmed, especially in Africa. Five human rabies deaths from Tanzania and Kenya were investigated and the causative rabies viruses sequenced, with the aim of identifying implications at individual, healthcare and societal levels”. (lines 35-38)*

1. Case presentation: re-write this section. Please summarize the exposure history of 5 cases in this section (such as 4 with a clear history of being bitten by dogs and 1 case with an unclear biting history), medical history (such as how many cases seeking medical attention on the same day or within 1 day after being bitten, and how many cases seeking medical attention after experiencing symptoms), and PEP history. Additionally, please move the phylogenetic investigation results in this part.

*We have rephrased the section to follow this guidance “The epidemiological context and care of these cases is contrasting: Four had a clear history of being bitten by dogs, while one had an unclear biting history. Two individuals sought medical attention within a day of being bitten, whereas three sought care only after developing symptoms. Despite seeking medical care, none of the cases received complete post-exposure prophylaxis: one patient received only tetanus vaccination, one did not complete their post-exposure vaccination regimen, one followed an off-label vaccination schedule, and two did not receive any post-exposure vaccinations before the onset of symptoms. These cases highlight serious gaps in health-seeking behaviour, competency of healthcare professionals in handling rabies exposures, and accessibility and effectiveness of post-exposure prophylaxis as it is administered in the region”. (lines 39-47)*

1. Conclusions: Please provide conclusions directly without "We conclude that...".

*We have addressed the comment and removed the word “we” from the conclusion.*

1. Keywords: Was "next-generation sequencing" applied in the research? If not, it should not included in the keywords. Besides, please consider whether "rabies" and "East Africa" suitable for keywords.

*We included "next-generation sequencing" as a keyword because Nanopore sequencing is a type of next-generation sequencing used for this study. We did not include "rabies," as a keyword since it already appears in the title and abstract. However, we added "East Africa" as suggested.*

4. Main text:

1. For the introduction of individual cases, it is recommended to follow a unified sequence, starting with the patient’s visit to the medical facility, then tracing back to the patient's exposure history, whether they sought medical attention after exposure and any related treatments, the history of the onset of the disease, and finally, the progression of the disease, treatment, and outcome after the visit. Alternatively, you can narrate in chronological order, starting from the exposure and proceeding in the order of time development.

*Thank you. We have rephrased the cases following the recommended unified sequence from case presentation with symptoms of rabies (lines 128-196*).

1. In the introduction of Case 1, the timeline is quite confusing. According to Table 1, the patient was bitten by a dog on January 23, 2018, developed symptoms 28 days later, and died 3 days after the onset of the disease. However, the introduction states that the symptoms appeared on February 23, which does not match the information in Table 1.

*It was reported that the patient was bitten by the dog a month prior to visiting the hospital, with the exact date of exposure not specified, only mentioned as approximately a month earlier. To ensure accuracy and avoid any confusion, we have updated the table and stated the uncertainty reported by the family “Approximately one month”.*

1. In the introduction of Case 2, it is mentioned that the patient sought medical attention on July 3, 2018, and passed away on July 8. However, Table 1 indicates that the patient visited the hospital on July 8, which is inconsistent information.

*Thank you for checking this thoroughly. The patient visited the hospital on 3rd July. We have corrected the dates in the table accordingly.*

1. In the introduction of Case 5, the patient was bitten by a dog on September 8, 2022, and transferred to another hospital for treatment on September 27, which should be 19 days later, not 20 days. Table 1 also shows a latency period of 19 days. The patient passed away on October 3, which would be 25 days after the bite and 6 days after the onset of the disease, not 26 days after the bite (as described in the main text) or 7 days after the onset of the disease (as shown in Table 1). Please verify the specific number of days and ensure consistency throughout the text.

*Thank you for the comment. We have corrected the number of days following the case details, both in the table and the main text.*

1. Please check the timelines of the other cases for any errors as well.

*We have cross checked the dates and number of days for cases 3 and 4 as well to ensure consistency.*

1. Discussion and Conclusions: In the first paragraph, you should state clearly the main conclusions, including an explanation of their relevance or importance to the field.

*We have updated the discussion and conclusions section to state our main conclusion in the first paragraph (moving up some of the text from the last paragraph), and an explanation of their importance.*

5. Figures:

1. In Figure 1, please label the three figures as A, B, and C respectively, and provide explanations for each in the figure captions. Additionally, please specify in the captions the type of tissue samples used and the magnification factor after rectangular amplification.

*We have added panel labels as advised and now specify in the captions the type of samples used and the magnification factor.*

1. Please move the maps from Figure 2 and Figure 3 to the supplementary materials, and consider combining the remaining parts into one figure. Additionally, please revise the figure captions, add sub-figure numbers (A, B, C, etc.), and update the image citations in the main text accordingly.

*The figures that have maps (2 and 3) were described positively by the reviewers. We purposely included the maps together with the phylogenies to illustrate the co-circulation of different lineages and their cross-border movement which was a key finding. For these reasons we have simplified both Figures but kept the East Africa map (Fig 2) and the zoomed in map (Fig 3), and we have updated the panel labels and legends to better convey the key messages from our work.*

1. Please ensure that the dates on the phylogenetic tree images are consistent with those in the main text, Table 1, and Supplementary Table 1.

*We have made sure that the dates on the phylogenetic tree images are consistent with those in the Supplementary Table 1.*

1. Please revise the words within figures according to the following font/size::

· Caption number: MyriadProUnic Bold (TrueType) l 8/10pt

· Content: MyriadProUnic Light (TrueType) l 8/10 pt

If this font is not available for you, please use Calibri or Arial.

*The font used is Arial.*

1. Please provide high quality images of the figures (image resolution of approximately 300 dpi (dots per inch) at the final size).

*We have uploaded high quality vector images for our resubmission.*

6. Supplementary file 1:

1. The title would be better if it also included geographical location analysis and phylogenetic analysis.

*Thank you for the suggestion. However, we did not do any geographical analysis of the data; the case locations (latitudes and longitudes) were used for maps to visualise the distances between cases from the same lineage. The title on file 1 is drafted to reflect the scope of our laboratory work from case diagnosis to sequencing.*

1. Please specify the sources of all the other samples besides 5 cases.

*We have updated the table to specify the sources of all additional samples included in the study. To distinguish between the samples sequenced in this study and those that are publicly available, we have used the italic format for the samples sequenced as part of this research.*

7.Supplementary Table 1:

1. In this table, the sequences of the 5 cases in this study should be distinguished.

*Thank you for the suggestion. The sequences of the 5 cases in the table have now been bolded for clarity.*

1. The values of 76.75 for Case 1 and 97.49 for Case 3 in Table 1 are not found here.

*Thank you for the observation. We have now corrected the genomic coverage with the right value.*

1. Why are some PubMed IDs missing? Are they the 99 new sequences? And there should be a sign like "－" or NA in the empty cells with an explanation in the annotation.

*We have added the PubMed IDs where available. Since the sequences generated from this study do not yet have a Pubmed ID we have indicated “This study” in the corresponding cells.*

8.Supplementary Table 2:

1. According to Supplementary Table 1, OR920307 is a sample from a dog in 2019, but Case 2 occurred in 2018. Should the animal sample come after the human sample? “Time between cases” should be a “+” instead of a “-”?

*Thank you for pointing this out. The metadata for this case in Supplementary table 1 was incorrect. This has now been corrected, with OR920307 in 2017; prior to case 2.*

9.If you want to show a range of values, such as data, time, date range, it would be better to use an en-dash (–).

*Thank you for the suggestion. We have used an en-dash (–) to indicate ranges such as dates, times, or data ranges in the text and figures.*

10. I made some minor changes in the main text and tables. More details are shown in the annotations of the article and supplementary materials. And please modify your manuscript based on this version.

*Thank you for the revisions. We reviewed the changes made to the main text and tables, and have modified the manuscript based on your annotations in the article and supplementary materials.*

**Other reviewer comments**

Other comments were provided by one of the reviewers as an attachment. We have outlined how we addressed these below:

1. On the case presentation “Please note the singular and plural forms of numbers referring to people”

*We have now corrected the grammar.*

1. Other treatment at the health facility section in table 1 - case 2, 4 and 5 - “Please be consistent with the text above”

*We have added another row which specifies the treatments provided for patients presenting with rabies symptoms for consistency.*

1. Genome coverage in table 1 - case 1 and 3 “Inconsistent with information in Supplementary table 1”

*Apologies for this error. We have corrected the genome coverage percentage.*

1. According to Supplementary file 1, “Sets of previously designed multiplex primer sets used in Tanzania from 2019-2020 [8] and updated to include the greater RABV diversity in Kenya (used from 2020-2023”. How to explain?

*The primer set was initially designed to cover RABV diversity in Tanzania. This primer set was used for sequencing some earlier samples in Tanzania and a few in Kenya including case 1 and 2. However, this initial design resulted in low genomic coverage for these two cases. With the inclusion of samples from Kenya, we developed a new set of primers to cover the diversity found in both countries, ensuring better coverage of the more recent samples.*

1. Referred sequences from Burundi, Kenya, Uganda, 2019 Serengeti, Ethiopia, Morocco and Algeria, are not found in Supplementary Table 1. Please check for any errors? If there are no errors, please explain the reason.

*Thank you for the comment. We have added the missing sequences to Supplementary Table 1 and corrected the results section to include the most recent data for comparison.*

1. Discussion “Table 1 shows that frozen samples were used for sequencing in Case 3, which is inconsistent with the information here.”

*A sample from case 3 was stored frozen in a freezer before being used for sequencing - this was the same sample that tested RDT negative. A formalin-fixed sample from the same case was used for immunohistochemistry (Fig 1). These details are indicated in Table 1.*