Version 0

Reviewers' comments:
Reviewer #1 (Remarks to the Author):

The manuscript addresses an important topic; however, several issues limit its contribution and clarity, particularly regarding timeliness, novelty, and sustainability of capacity building.

1) Timeliness and Updates (Lines 112–114)

The manuscript notes that initial sampling of suspected mpox cases began around a local hospital in late 2023 that lacked diagnostic capacity and adequate infrastructure. Given that this work is presented in 2025, an update is expected.

- Have diagnostic, sampling, storage, or referral capacities improved since 2023?

- If so, these developments should be described and analysed.

Without such updates, the description feels static and reduces the manuscript's contemporary relevance.

2. Lack of Clarity on Collaborations (Line 110)

The manuscript refers to activities conducted "through our collaborations," but these collaborations are not clearly defined.

- Please specify the collaborating institutions or networks, their respective roles, and how these partnerships contributed meaningfully to capacity building.

- Clarifying whether these collaborations represent a new or strengthened model would enhance transparency and credibility.

3. Well-Known Constraints Without Added Insight (Lines 200–206)

The discussion on limited access to Nanopore sequencing consumables and the suggestion of local production or subsidisation reflects widely recognised challenges in genomic surveillance.

- The manuscript would benefit from additional analysis, such as feasibility

considerations, pilot experiences, or country-specific pathways, rather than reiterating known constraints.

- As written, this section restates the problem without advancing the discussion.

4. Repetition of Previously Published Arguments (Lines 227–229)

The authors argue that sustainable and long-term capacity building requires longer programmes and collaborations under limited funding. This point closely mirrors conclusions previously published by previous authors (Agboli et al., Trop. Med. Infect. Dis., 2025), where training duration—particularly for bioinformatics—was identified as the main limiting factor.

- In the current manuscript, this argument is repeated but not clearly extended or supported by new evidence.

- The authors should clarify what new insight this manuscript adds beyond earlier publications.

5. Strengthening the Contribution

Overall, the manuscript would be strengthened by:

- Clearly identifying what is new compared to prior work;

- Demonstrating how the described activities contribute to institutionalised, sustainable capacity building, rather than short-term or project-based interventions;

- Providing updated outcomes or lessons learned since initial implementation phases.

Reviewer #2 (Remarks to the Author):

In the title, I would like to introduce the term Mpox, since you talked a lot about Mpox in the manuscript 'Capacity building for genomic surveillance of Mpox and other emerging diseases in resource-limited settings in the African Great Lakes region'.
The Great Lakes region, particularly the DRC, is an area marked by the emergence of new strains or clades of several pathogens as Mpox.
Au niveau de la ligne 32, Genomic surveillance has become an essential and INDISPENSABLE tool for identifying pathogens.
In your context, I think it is important to mention the high population density in this area

and the intensity of inter- and cross-border movements, which also facilitate the high migration or spread of pathogens. This means that the genomic diagnosis of microbes in general, and those responsible for emerging diseases in particular, must be treated as a public health emergency and form the basis of a sustainable surveillance system.
In the context of line 48, it would be better for me to say characterisation of epidemic STRAINS.
At line 50, ... of this genomic surveillance TECHNIQUE has facilitated immediate risk assessments.
In LMICs, health infrastructure is outdated and poorly maintained. Added to this is the problem of electricity, although palliative measures are being taken with solar fields equipped with photovoltaic panels and lithium batteries. The difficulty lies in the supply of biomedical equipment and the training or even retraining of health personnel working in these sometimes very remote areas.
The important thing is to develop molecular diagnostic capabilities. To do this, laboratory staff need to be trained in standard operating procedures (SOPs), interpreting and reporting results, the reliability of results, and sequencing, which is an important step in identifying the genetic signature of the microbe in question.
In LMICs in the Great Lakes region, sequencing has been facilitated, as you so aptly wrote, by the arrival of nanopores, a portable, low-cost and accessible technology. However, cold chains are essential, especially for the supply and storage of often scarce reagents and samples. Bioinformaticians, whom you should discuss at length in your analysis of genomic data, enable a reduction in response time between sampling and health action.
Genomic surveillance is not simply a diagnostic tool, but a technical and biological safety imperative with the One Health approach, which brings together human, animal and environmental health, facilitating a multidisciplinary team approach while also facilitating a decentralised genomic molecular diagnostic approach.

Version 1

Reviewers' comments:
Reviewer #1 (Remarks to the Author):

The authors have done an excellent job addressing the remarks from the first round of reviews. The revisions are comprehensive, and the additional clarification provided has significantly increased the rigor of the manuscript.

Therefore, with the added context in the introduction, the novelty of the findings is now well-justified and will certainly interest the wider community with the authors providing the necessary further evidence and references requested. This now reinforces the

primary conclusions.

I believe the paper will be influential in shifting the perspective on "Capacity building for genomic surveillance of emerging diseases in resource-limited settings within the African Great Lakes region"

I am satisfied with the current version of the manuscript and recommend it for publication without further delay.

Reviewer #2 (Remarks to the Author):

Thank you for allowing me to contribute to your manuscript.
Genomic surveillance is becoming a key element in disease control and prevention.

Abstract
I would ask you to revise the abstract slightly to reflect the contributions and suggestions
I have made

Background
I shall begin by asking you to include in your background section a description of the location of the African Great Lakes region and the countries that make it up. This will immediately provide readers—whether scientific or not—with an introduction to this particular and complex region and its assets. It should also be noted that this region is the epicentre of certain pathogens that have spread worldwide as epidemics, a hotspot for biodiversity and the emergence of zoonotic diseases, where it is important to move from a reactive response following several deaths to proactive prevention through microbial surveillance, including a One Health approach.

Although your arguments revolve around a context of limited resources, the issue here is not about acquiring or purchasing the latest high-tech sequencing machines, but rather about having sustainable capabilities to address any threats before a pandemic situation takes hold.

When comparing the 17% of SARS-CoV-2/GISAID sequences from the UK for a population of 69.2 million with the 1% of SARS-CoV-2/GISAID sequences from the African continent for a population of 1.4 billion, you should also have highlighted the paramount importance of peace, health, education and the common good. What are the political priorities in this region?

Admittedly, the situation has improved significantly since the Ebola outbreak in Sierra Leone in 2015, and this improvement has been further reinforced by the COVID-19 pandemic in 2020. Furthermore, it would be worth noting the resilience demonstrated by the development of a versatile local capacity to limit the need to send samples abroad, which was secondary to increased biological risks and the extreme wariness of airlines regarding the transport of pathogens in any form.

Developing capabilities in molecular testing AND sequencing, cost-effectiveness, supply chains and data analysis
In this section, I would suggest supplementing this paragraph with, on the one hand, “a suitable infrastructure featuring technologies such as point-of-care sequencers – which you mentioned, such as Nanopore and portable devices – cold chains and logistics to ensure the safety of reagents, samples and vaccines, utilising in particular solar energy, which is now widely available, and finally regional centres for more advanced analysis; and, secondly, a well-trained and equipped workforce in molecular analysis (characterisation), bioinformatics and other fields. Training for biologists and researchers
is essential in order to utilise data pipelines, provide clear epidemiological information and identify anomalies or variations—such as protein mutations—responsible for variants and new outbreaks.

As you mention POCs such as GeneXpert Mpox (Cepheid), it would be wise to make these
available in all health districts or prefectures, along with a minimum stock of test cartridges, in order to be prepared for any eventuality

Personal reflections, futures directions and concluding remarks
All these tools and techniques must integrate genomics into epidemiological surveillance
and contribute to the public health debate, so as to enable real-time, effective surveillance that links patients’ clinical data with sequencing data in order to map diseases at the very moment of an outbreak; to provide early warnings through the rapid,
accelerated identification of any pathogenic strain, its mutations, virulence and resistance to existing drugs.

In this region, populations frequently cross borders. This highlights the importance of multi-party agreements on data sharing, removing political barriers on ethical and sovereignty grounds so that data and study results can be used first and foremost for the
benefit of the local population and subsequently the international community. It would

be important to ensure biological and IT security by preventing biopiracy by hackers. Public health emergencies of international concern (PHEICs) are frequent in this region, but people are mostly prone to endless discussions that lead to no concrete action, thereby facilitating the rapid spread of the disease and the microbe responsible for it.

Another point I would suggest you include in your paper is to include some text highlighting the importance of local scientists who are capable of carrying out "integrated research on epidemics, to establish pre-approved protocols enabling a rapid research response and to write scientific articles, which are essential for laying the foundations to maximise the impact on public health", as all too often those who are capable are sidelined because of their ideology and principles.

In your last paragraph, a word was missing from the sentence, and I have highlighted it in capital letters: "It is crucial to recognise current differences in capacity and to highlight the challenges faced in LMICs to help develop and sustain genomic surveillance, thereby strengthening decision-making at the national, regional and global levels."

In these Great Lakes countries, the interaction between humans, animals and the environment is constant, necessitating cross-cutting genomic surveillance within a One Health approach.

Thank you

Version 2

Reviewers' comments:
Reviewer #2 (Remarks to the Author):

The Great Lakes region is the epicentre of numerous pathogens responsible for several epidemics worldwide, and a hotspot for biodiversity and the emergence of zoonotic diseases. The key is to move from a reactive response following several deaths to proactive prevention by monitoring the microbe using the One Health approach. This involves building robust local capacity through the training of biologists and researchers capable of delivering results rapidly using genomic diagnostic and surveillance techniques.

Reviewer #1 (Remarks to the Author):

The manuscript addresses an important topic; however, several issues limit its contribution and clarity, particularly regarding timeliness, novelty, and sustainability of capacity building.

We would like to thank the reviewer for their feedback and for reviewing of this manuscript.

1) Timeliness and Updates (Lines 112–114)

The manuscript notes that initial sampling of suspected mpox cases began around a local hospital in late 2023 that lacked diagnostic capacity and adequate infrastructure. Given that this work is presented in 2025, an update is expected
- Have diagnostic, sampling, storage, or referral capacities improved since 2023?
- If so, these developments should be described and analysed.

Without such updates, the description feels static and reduces the manuscript's contemporary relevance.

We thank the reviewer for highlighting the need to more explicitly update the diagnostic and operational context since the beginning of capacity building efforts and emergence of MPXV in late 2023. The developments and improvements might sound static sometimes as we tried to keep descriptions general and focused on capacity development rather than attributing a single site or actor in the manuscript.

We have merged the paragraph highlighted by the Reviewer with the subsequent paragraph to make the connection more clear. This restructuring helps to connect on how the initial contextual sentence relates to the general recommendations described in the following section: (i) safe sampling and biosafety practice through targeted training (Line 122), (ii) sample preservation and transport strategies through the introduction of established preservative approaches such as virus transport medium (Line 136-139), and (iii) continued piloting of field-adapted preservation methods including activated charcoal and SDS cards (Line 140). We have now also added a description of strengthened cold chain/ storage capacity through access to centralized freezer storage (Line 142).

In addition, we clarified that diagnostic capacity expanded through the application of open-platform real-time PCR assays for MPXV detection (Lines 163–166), with the approach being extendable to other pathogens. Finally, we added details on decentralised sequencing capacity building: MinION devices, reagents, and laptops were distributed to local partners, and on-site training combined with remote support enabled independent local sequencing in local hubs (Huye, Rwanda; Bukavu, South

Kivu; and Bujumbura, Burundi). Together, these developments strengthened referral/confirmation workflows and enabled local generation of genomic data to support MPXV surveillance following emergence in DRC and detection in neighbouring countries. For example in Burundi, clade assignment of the first detected mpox cases was generated using GREAT LIFE reagents/protocols and locally implemented sequencing workflows, demonstrating that infrastructure (equipment, protocols, and trained personnel) is now in place to support ongoing genomic surveillance.

Examples of application on initial work and application of monitoring and characterizing of MPXV in neighbouring country include Nzoyikorera et al. (2025, DOI:10.1038/s43856-025-01199-6), Schuele et al. (2024, DOI:10.2807/1560-7917.ES.2024.29.32.2400486), and Nzoyikorera et al. (2024, DOI:10.2807/1560-7917.ES.2024.29.42.2400666). More published work from our consortium and used protocols are listed on https://www.globalsurveillance.eu/projects/great-life, which we have now also included into the manuscript based on the Reviewer's next suggestion (see below).

2. Lack of Clarity on Collaborations (Line 110)

The manuscript refers to activities conducted "through our collaborations," but these collaborations are not clearly defined.

- Please specify the collaborating institutions or networks, their respective roles, and how these partnerships contributed meaningfully to capacity building.
- Clarifying whether these collaborations represent a new or strengthened model would enhance transparency and credibility.

We agree that "through our collaborations" might need a clearer definition. We have clarified this in the paragraph in Line 91 by referring to the GREAT-LIFE framework and the JUA KIVU network (Refs. 6,7) and now added the links (https://www.globalsurveillance.eu/projects/great-life) directly into the manuscript. We reintroduced these references again at Line 106 to make the link between the statement and the collaboration clearer. Also, in lines 117-188 we have now added "The studies conducted through our collaborations, established in the GREATLIFE consortium and strengthened after the formation of the JUA KIVU consortium,". Permanent digital object identifiers (doi) and website of the project, as well as the websites are now listed in the manuscript.

The roles of the partners have been described on the project pages which we link to. These pages contain full partner lists, institutional contact information, and documentation of outputs of the consortium (publications, training/workshops, protocols, and ongoing sampling activities). In short, local clinical sites and public health/lab hubs coordinate case identification and sampling. Laboratories perform

confirmatory diagnostics and sequencing. Academic/international partners provide protocol development and training with follow up mentorship and support with data analysis and reporting. Those initial capacity training tasks were mainly performed by the Erasmus Medical Center (viral diagnostics) and by the Danish Technical University (bacterial characterization and user friendly bioinformatic pipelines).

3. Well-Known Constraints Without Added Insight (Lines 200–206)

The discussion on limited access to Nanopore sequencing consumables and the suggestion of local production or subsidisation reflects widely recognised challenges in genomic surveillance.

- The manuscript would benefit from additional analysis, such as feasibility considerations, pilot experiences, or country-specific pathways, rather than reiterating known constraints.

- As written, this section restates the problem without advancing the discussion.

The limited access to Nanopore consumables is indeed still a well-known constraint. Because availability and procurement pathways differ across countries and settings, and because we aim to avoid endorsing specific vendors, e.g. https://nanoporetech.com/about/channel-partners, the manuscript remains general in brand naming. However, we describe a few different logistical approaches which could be applied to others to help to address these limitations (of reagents).

Specifically, we added text describing how procurement can be operationalized through coordination among local partners with centralized storage (this is the way to go) and on the other hand shipment routing via large international airports (e.g., Kigali International Airport and Melchior Ndadaye International Airport, Bujumbura) followed by local distribution. We also explicitly note why shipment via airports is not a long-term solution, as it adds significant organizational complexity, increases costs, lead times and customs delays can compromise availability and functionality of reagents. To make the feasibility requirements clearer, we also highlight basic infrastructure needs such as reliable -20°C storage (widely available and usable for both samples and reagents), which is crucial for maintaining reagent integrity during storage and distribution. We have highlighted this more in the revised manuscript and added the sentence “The acquisition/presence of a centralized -20` C freezer for (acute and prospective) sample and reagent storage also played a crucial role in prospective workshops.” (Line 142-144).

To move beyond constraints, we also highlighted the feasibility of adapting workflows to local availability, including the use of alternative reagents for Nanopore workflows

(Kovalenko et al., dx.doi.org/10.17504/protocols.io.36wgqqzq5gk5/v1; Reference 19), which we found extremely useful when implementing sequencing on site. We have made this a little more clear by changing the previous sentence in the manuscript "Another option is the substitution of certain reagents with alternative and commonly stocked enzymes and buffers and to implement those within existing sequencing workflows" to "Another option is the substitution of certain reagents with alternative or commonly stocked enzymes which can be implemented into existing sequencing workflows" (Line 231-234).

Finally, we clarify that strengthening local diagnostics and sequencing capacity helps demonstrate sustained demand and feasibility, which is a necessary precondition for country-/region-specific logistics such as pooled procurement, regional distribution hubs, or longer-term local manufacturing initiatives, rather than presenting local production as an immediate solution.

4. Repetition of Previously Published Arguments (Lines 227–229)

The authors argue that sustainable and long-term capacity building requires longer programmes and collaborations under limited funding. This point closely mirrors conclusions previously published by previous authors (Agboli et al., Trop. Med. Infect. Dis., 2025), where training duration—particularly for bioinformatics—was identified as the main limiting factor.
- In the current manuscript, this argument is repeated but not clearly extended or supported by new evidence.

- The authors should clarify what new insight this manuscript adds beyond earlier publications.

The call for the need of longer-term training and sustained collaborations is indeed consistent with earlier observations, including Agboli et al. (2025), where bioinformatics training duration was highlighted as a key limiting factor. Therefore we added some more information regarding these points.

We noticed during the training there was limited basic computational knowledge for instance on cloud computing and on different operating systems through which it was not easy to use a common procedure/guideline for all participants and more specific/dedicated long term training is needed to make the capacity building processes more durable.

After the first training, the focus shifted from initial wet-lab foundations (with sequencing now performed locally) toward increasing emphasis on dry-lab skills, as also requested by the participants, and we highlight that GUI-oriented workflows can help more users participate in analysis compared with purely command-line

approaches. Both the GUI based programs we use and recommend (Table 1) run on several operating systems (Windows, Linux/mac OS). We have introduced this into the paragraph in Lines 223 -226 which now state “Based on our experiences, the training focus shifted from initial wet-lab foundations (with sequencing now performed locally) towards increasing emphasis on dry-lab skills. Here the application of GUI based workflows (Table 1) can help more users participate in analysis compared with purely command-line approaches”. We have linked additionally to Table 1 in the text again which lists analysis options and the functional features we consider important (e.g., usability, reproducibility, and reduced dependency on command-line expertise).

Within GREAT-LIFE, collaborators at DTU developed CGELabs (referenced in Table 1, also added another link in Line 279), as an example of a non-commercial tool built to support the capacity-building goals. Finally, we have added Agboli et al. as a reference within this section (Line 237).

5. Strengthening the Contribution

Overall, the manuscript would be strengthened by:
- Clearly identifying what is new compared to prior work;
- Demonstrating how the described activities contribute to institutionalised, sustainable capacity building, rather than short-term or project-based interventions;
- Providing updated outcomes or lessons learned since initial implementation phases.

This perspective concatenates our recent insights from ongoing capacity building in the region during outbreak and non-outbreak settings, from sampling and storage constraints through diagnostics, sequencing, and analysis.

We have strengthened aspects in the manuscript on how the described ongoing activities contributed to capacity building beyond a single study:

Establishment of local sequencing capability inside and outside national reference centres; procurement and use of shared infrastructure (-25°C/-20°C storage for samples and reagents) (Line 140-143); introduction of MPXV real-time PCR and clade differentiation in centralized local settings with extension to the principle to other targets to strengthen laboratory capacity development (Lines 163 - 165); repeated and ongoing trainings with progressive shift from wet-lab foundations to sustained dry-lab capacity with program/approached based suggestions for user friendly bioinformatic analysis (Lines 278-280).

We hope these clarifications address the reviewer’s remarks and enhance the understanding of the scope and limitations.

Reviewer #2 (Remarks to the Author):

In the title, I would like to introduce the term Mpox, since you talked a lot about Mpox in the manuscript 'Capacity building for genomic surveillance of Mpox and other emerging diseases in resource-limited settings in the African Great Lakes region'. The Great Lakes region, particularly the DRC, is an area marked by the emergence of new strains or clades of several pathogens as Mpox.

We would like to thank the reviewer for their feedback and reviewing of this manuscript.

We have included the Reviewer's suggestion into the manuscript. The title reads now as: "Capacity building for genomic surveillance of mpox and other emerging diseases in resource-limited settings in the African Great Lakes region".

Au niveau de la ligne 32, Genomic surveillance has become an essential and INDISPENSABLE tool for identifying pathogens.

We believe that there is quite an overlap between the words essential and indispensable in our opinion. As the abstract should be as concise as possible, we would like to keep it as is and hope the content reitaerates the indispensable aspect of genomic surveillance for identifying pathogens.

In your context, I think it is important to mention the high population density in this area and the intensity of inter- and cross-border movements, which also facilitate the high migration or spread of pathogens. This means that the genomic diagnosis of microbes in general, and those responsible for emerging diseases in particular, must be treated as a public health emergency and form the basis of a sustainable surveillance system.

We have added the following sentence in Lines 92-96 to set the region into perspective when discussing the need to strengthen surveillance: "The African Great Lakes region is one of the most densely populated and biodiverse areas in Africa, with significant levels of inter- and cross-border movements that can facilitate the emergence and spread of pathogens, making genomic surveillance and diagnosis a public health priority."

In the context of line 48, it would be better for me to say characterisation of epidemic STRAINS.

We have incorporated the Reviewers suggestion into the sentence in line 50, which now reads "During the COVID-19 pandemic, genomics was applied at an unprecedented scale for global surveillance, monitoring spillover events, characterizing epidemic strains and detecting new variants with possible phenotypic changes".

At line 50, … of this genomic surveillance TECHNIQUE has facilitated immediate risk assessments.

We have incorporated your suggestion into the sentence which now reads: “The real-time application of this genomic surveillance *technique* facilitated input for immediate risk assessments” (Line 51).

In LMICs, health infrastructure is outdated and poorly maintained. Added to this is the problem of electricity, although palliative measures are being taken with solar fields equipped with photovoltaic panels and lithium batteries. The difficulty lies in the supply of biomedical equipment and the training or even retraining of health personnel working in these sometimes very remote areas.

Very true. We have touched upon this in the manuscript and find it is particularly crucial to use a backup battery for real-time PCR (Table 1). We also list the BentoLab and Nanopore sequencing with laptops to bridge power cuts.

The important thing is to develop molecular diagnostic capabilities. To do this, laboratory staff need to be trained in standard operating procedures (SOPs), interpreting and reporting results, the reliability of results, and sequencing, which is an important step in identifying the genetic signature of the microbe in question.

Indeed and in our consortium we publish the applied protocols, the link we have now also directly linked in the manuscript based on the Reviewer’s comment (https://www.globalsurveillance.eu/projects/great-life/great-life-protocols) (Line 106) and data on public databases. The capacity building workshops started with focus on wet lab with increasing shift to data analysis when wet lab protocols are in place on site.

In LMICs in the Great Lakes region, sequencing has been facilitated, as you so aptly wrote, by the arrival of nanopores, a portable, low-cost and accessible technology. However, cold chains are essential, especially for the supply and storage of often scarce reagents and samples. Bioinformaticians, whom you should discuss at length in your analysis of genomic data, enable a reduction in response time between sampling and health action.

We have reiterated and added details that made the consortium acquire two -20/-25`C freezers that can be used for both acute and prospective sampling campaigns as well as the separate storage of reagents (Lines 142-144).

We have reinforced the time reduction aspect in Lines 274-275 “Local bioinformatics capacity is essential for independent pathogen surveillance and for shortening the interval between sampling and public health decision-making”.

After the first training, the focus shifted from initial wet-lab foundations (with sequencing now performed locally) toward increasing emphasis on dry-lab skills, as also requested by the participants, and we highlight that GUI-oriented workflows can help more users participate in analysis compared with purely command-line approaches. Both the GUI based programs we use and recommend (Table 1) run on several operating systems (Windows, Linux/mac OS). We have introduced this into the paragraph in Lines 223 -226 which now state “Based on our experiences, the training focus shifted from initial wet-lab foundations (with sequencing now performed locally) towards increasing emphasis on dry-lab skills. Here the application of GUI based workflows (Table 1) can help more users participate in analysis compared with purely command-line approaches”. We have linked additionally to Table 1 in the text again which lists analysis options and the functional features we consider important (e.g., usability, reproducibility, and reduced dependency on command-line expertise).

Genomic surveillance is not simply a diagnostic tool, but a technical and biological safety imperative with the One Health approach, which brings together human, animal and environmental health, facilitating a multidisciplinary team approach while also facilitating a decentralised genomic molecular diagnostic approach.

We agree that genomic surveillance should not be framed solely as a diagnostic tool, but also as a preparedness and biological-safety imperative. We believe strengthening laboratory capacity (training, workflows and infrastructure) is a critical pillar of this. In One Health activities, our consortium has also initiated wastewater (sewage) surveillance as a complementary approach to monitor community-level virus circulation (https://juakivu.org/news/sampling/).

Reviewer #1 (Remarks to the Author):

The authors have done an excellent job addressing the remarks from the first round of reviews. The revisions are comprehensive, and the additional clarification provided has significantly increased the rigor of the manuscript.

Therefore, with the added context in the introduction, the novelty of the findings is now well-justified and will certainly interest the wider community with the authors providing the necessary further evidence and references requested. This now reinforces the primary conclusions.

I believe the paper will be influential in shifting the perspective on "Capacity building for genomic surveillance of emerging diseases in resource-limited settings within the African Great Lakes region"

I am satisfied with the current version of the manuscript and recommend it for publication without further delay.

We would like to thank the reviewer again for their valuable feedback and reviewing of this manuscript.

Reviewer #2 (Remarks to the Author):

Thank you for allowing me to contribute to your manuscript.
Genomic surveillance is becoming a key element in disease control and prevention.

We would like to thank the reviewer again for their valuable feedback and reviewing of this manuscript.

Abstract
I would ask you to revise the abstract slightly to reflect the contributions and suggestions I have made

We have now incorporated the reviewers previous remark (addition of the word indispensable) into the abstract of the manuscript which now reads: “Genomic surveillance has become an indispensable tool for the identification of pathogens and tracking of transmission chains”.

Background
I shall begin by asking you to include in your background section a description of the location of the African Great Lakes region and the countries that make it up. This will

immediately provide readers—whether scientific or not—with an introduction to this particular and complex region and its assets.

It should also be noted that this region is the epicentre of certain pathogens that have spread worldwide as epidemics, a hotspot for biodiversity and the emergence of zoonotic diseases, where it is important to move from a reactive response following several deaths to proactive prevention through microbial surveillance, including a One Health approach.

This is a valuable suggestion to add geographic context for the reader. We have added information on the location of the African Great Lakes region and the countries that are part of our capacity building effort in (Lines 89-94):

"The African Great Lakes region includes a series of lakes and the surrounding land area in and around the East African Rift. In a narrow sense and within the regions of this perspective it encompasses Burundi, the Democratic Republic of the Congo (DRC), Rwanda and Tanzania. This grouping is characterized not only by shared geography but also by intense human mobility and trade via the Central Corridor transport network."

We have further included the following sentence into last revision which follows the reviewer's suggestion (Lines 94-98):

"The African Great Lakes region is one of the most densely populated and biodiverse areas in Africa, with significant levels of inter- and cross-border movements that can facilitate the emergence and spread of pathogens, making genomic surveillance and diagnosis a public health priority."

Although your arguments revolve around a context of limited resources, the issue here is not about acquiring or purchasing the latest high-tech sequencing machines, but rather about having sustainable capabilities to address any threats before a pandemic situation takes hold.

We appreciate the reviewer's perspective on the importance of sustainable capabilities. The choice of Nanopore, specifically the portable MinION devices (Mk1b & Mk1d), was not solely a cost-saving measure but also a strategic decision to ensure long-term operational sustainability in resource-constrained environments.

Unlike centralized, high-throughput platforms that require high throughput and come with high maintenance costs, the MinION platform enables decentralized routine surveillance. This facilitates local laboratories to maintain capabilities ensuring they are prepared to detect emerging threats before they become pandemics. Furthermore, the MinIONs Mk1m / Mk1d connectivity to laptops as power source (and data analysis platform) address common infrastructure failures (like power surges or power cuts) that

can often be a challenge for other platforms. By lowering the barrier to entry for both cost and training, this approach can foster local workforce retention and scientific independence both crucial parts of a sustainable public health system.

When comparing the 17% of SARS-CoV-2/GISAID sequences from the UK for a population of 69.2 million with the 1% of SARS-CoV-2/GISAID sequences from the African continent for a population of 1.4 billion, you should also have highlighted the paramount importance of peace, health, education and the common good. What are the political priorities in this region?

We agree with the reviewer that technical metrics, such as whole genome sequencing rates, are influenced by their sociopolitical and socioeconomic contexts. However, a detailed socioeconomic analysis lies beyond the primary scope of this Perspective, which is aimed at sharing our consortium's technical experiences and providing recommendations for genomic surveillance in resource-limited settings.

We have added a small nod to the socioeconomic contexts in Lines 62-64 “Although this discrepancy is staggering, it reflects not only technical gaps but also different priorities as well as broader structural and socioeconomic challenges inherent to the region. Nevertheless, sequencing capacity has improved within recent years due to investments during the COVID-19 pandemic [2]

Admittedly, the situation has improved significantly since the Ebola outbreak in Sierra Leone in 2015, and this improvement has been further reinforced by the COVID-19 pandemic in 2020. Furthermore, it would be worth noting the resilience demonstrated by the development of a versatile local capacity to limit the need to send samples abroad, which was secondary to increased biological risks and the extreme wariness of airlines regarding the transport of pathogens in any form.

We thank the reviewer for bringing up this practical aspect, which we have now also in cooperated in Lines 82-85 “Recently, new capacity-building programs have been initiated to support institutions in countries that previously lacked sequencing capabilities, thereby bypassing the biological risks and logistical hurdles (e.g., airline restrictions) associated with international pathogen transport and accelerating genomic surveillance.”

Developing capabilities in molecular testing AND sequencing, cost-effectiveness, supply chains and data analysis
In this section, I would suggest supplementing this paragraph with, on the one hand, “a suitable infrastructure featuring technologies such as point-of-care sequencers – which you mentioned, such as Nanopore and portable devices – cold chains and logistics to

ensure the safety of reagents, samples and vaccines, utilising in particular solar energy, which is now widely available, and finally regional centres for more advanced analysis; and, secondly, a well-trained and equipped workforce in molecular analysis (characterisation), bioinformatics and other fields. Training for biologists and researchers is essential in order to utilise data pipelines, provide clear epidemiological information and identify anomalies or variations—such as protein mutations—responsible for variants and new outbreaks.

We thank the reviewer for these insightful suggestions regarding infrastructure and workforce development. We have addressed these points as follows:

Infrastructure and Solar Energy: We agree that cold chains are paramount. As noted in the previous revision (Lines 134-136), we highlighted the role of centralized -20°C storage. Furthermore, we have now added text in the Personal Reflections section (Lines 265-268) acknowledging the potential of solar energy and battery-buffered systems to mitigate power instability during transport and analysis.

Regional Centres: Our perspective emphasizes a decentralized model that remains supported by regional hubs for bioinformatics and quality control, aligning with the reviewer's suggestion for tiered analysis.

Workforce Development: We have expanded on the necessity of a multi-disciplinary workforce. While we previously highlighted bioinformatics (Lines 213-230), we have clarified that training should encompass usage of the entire bioinformatic pipelines from molecular characterization to the interpretation of protein mutations and variants to provide actionable epidemiological information (see also below).

Data Pipelines: We continue to advocate for both specialized training (e.g., regional programmes such as PHA4GE and VEME) and the use of accessible GUI (graphical user interface)-based tools to ensure that local biologists can identify anomalies and variations in real-time.

As you mention POCs such as GeneXpert Mpox (Cepheid), it would be wise to make these available in all health districts or prefectures, along with a minimum stock of test cartridges, in order to be prepared for any eventuality

We thank the reviewer for highlighting the role of POC diagnostics in decentralized preparedness. We agree that the widespread availability of platforms and cartridges at the district level is a significant goal for rapid case detection that is also the foundation for follow up genomics. We have added a sentence to the manuscript (Lines 149-151 "The expansion of POC networks to all health districts with basic stock of test cartridges is often advocated as a cornerstone of pandemic preparedness.") to acknowledge this strategy.

We have also maintained the drawbacks of POC platforms in our original discussion regarding the limitations of this approach, specifically concerning cost, the need of pathogen or syndrome specific cartridges, the inability to differentiate lineages and the lack of contribution to local workforce training, as these factors are central to our perspective on building sustainable, long-term genomic surveillance capacity.

Personal reflections, futures directions and concluding remarks

All these tools and techniques must integrate genomics into epidemiological surveillance and contribute to the public health debate, so as to enable real-time, effective surveillance that links patients' clinical data with sequencing data in order to map diseases at the very moment of an outbreak; to provide early warnings through the rapid, accelerated identification of any pathogenic strain, its mutations, virulence and resistance to existing drugs.

We thank the reviewer for outlining this vision for the future of public health. We agree that the ultimate utility of pathogen genomics lies in its seamless integration with clinical and epidemiological data to provide real-time early warnings. This requires a complex data ecosystem that goes beyond wet and dry lab capacity. Establishing such interlinked networks necessitates the development of robust legal and ethical frameworks for data protection and cross-border sharing which need foundations led by regional organizations, such as the Africa CDC.

We have added ", secure and privacy preserving data sharing protocols" (Lines 271-272) to the outlook as this would be the first step for linking patient and genomic data in this vision

In this region, populations frequently cross borders. This highlights the importance of multi-party agreements on data sharing, removing political barriers on ethical and sovereignty grounds so that data and study results can be used first and foremost for the benefit of the local population and subsequently the international community. It would be important to ensure biological and IT security by preventing biopiracy by hackers. Public health emergencies of international concern (PHEICs) are frequent in this region, but people are mostly prone to endless discussions that lead to no concrete action, thereby facilitating the rapid spread of the disease and the microbe responsible for it.

We thank the reviewer for highlighting the critical intersections of data sovereignty, biosecurity and regional cooperation. We agree that in a region with high population mobility, establishing multi-party agreements that prioritize the benefit of local populations is essential to move from discussion to concrete public health action.

We believe that building local genomic capacity is a prerequisite for this sovereignty, as it allows for the characterization of pathogens within the region of origin.

To address the reviewer's points on security and ethical data use, we have added our previous remark on secure data sharing protocols (Line 271-272). These additions underscore the importance of protecting regional health data while ensuring it serves as a tool for rapid, local outbreak response."

Another point I would suggest you include in your paper is to include some text highlighting the importance of local scientists who are capable of carrying out "integrated research on epidemics, to establish pre-approved protocols enabling a rapid research response and to write scientific articles, which are essential for laying the foundations to maximise the impact on public health", as all too often those who are capable are sidelined because of their ideology and principles.

We thank the reviewer for this insightful comment. We agree that the success of genomic surveillance relies on the leadership and scientific independence of local experts. We believe that providing local scientists with the tools to lead research is essential for moving toward a merit-based system that avoids the sidelining of capable individuals.

We have addressed this in the manuscript (Line 243-246) by explicitly stating that capacity building must include: time to train local scientists in embedded outbreak research, to set up protocols with pre-approval to allow rapid research response and to develop scientific papers which are important to lay the foundation to maximize public health impact.

By emphasizing embedded research and leading scientific publications, we advocate for a framework where local scientists are established in the public health response. We believe this existing text aligns with the reviewer's call to prioritize local scientific voices and ensure their expertise translates into documented public health impact.

In your last paragraph, a word was missing from the sentence, and I have highlighted it in capital letters: "It is crucial to recognise current differences in capacity and to highlight the challenges faced in LMICs to help develop and sustain genomic surveillance, thereby strengthening decision-making at the national, regional and global levels."

We thank the reviewer for catching this omission. We agree that strengthening decision-making at the national level is the primary goal of these efforts. We have updated the final sentence to include the national level which now reads (Lines 279-281):

“Recognizing current capacity differences and highlighting the challenges in LMICs is crucial to help expand and sustain genomic surveillance, thereby strengthening national, regional and global decision-making.”

In these Great Lakes countries, the interaction between humans, animals and the environment is constant, necessitating cross-cutting genomic surveillance within a One Health approach.

Thank you

We thank the reviewer for this final and important observation. We agree that the quite unique ecological context of the Great Lakes region necessitates a One Health approach. We have updated the manuscript to highlight how redirecting genomic capacity toward a broader range of zoonotic and emerging pathogens supports cross-cutting surveillance at the human-animal-environment interface (Line 74-77): “In these Great Lakes countries, the interaction between humans, animals and the environment is constant, necessitating integrated genomic surveillance within a One Health approach.”