Virology Journal

BRIEF REPORT

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A retrospective study revealing complex viral diversity and a substantial burden of HPV infection in SARS-CoV-2 positive individuals, Sierra Leone

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Abstract

Background The COVID-19 pandemic has underscored the critical role of sequencing technology in disease control and outbreak response. However, resource limitations and challenging environments often impede such efforts in low and middle-income countries. This study aimed to investigate the spectrum of viral co-infections, particularly with human viral pathogens, in SARS-CoV-2 positive individuals in Sierra Leone using metagenomic sequencing, evaluating the feasibility of utilizing this technology for epidemiological and evolutionary surveillance of pathogens related to public health in low-income environments.

Methods We retrospectively collected and analyzed 98 nasopharyngeal swab specimens from SARS-CoV-2 positive individuals in Sierra Leone. Samples were pre-processed locally and transferred to China via FTA cards for metagenomic sequencing, which was performed using the Novaseq platform. The study focused on the identification of nasopharyngeal viruses co-infecting with SARS-CoV-2, with a deeper analysis of significant human viral pathogens such as HPV.

Results The study identified 22 viral taxa from 20 families, including 4 human viruses. Notably, 19.4% of samples showed HPV co-infection with 34 distinct types, predominantly beta and gamma HPVs. Multiple HPV types were found in individual samples, indicating a high complexity of viral co-infections.

Conclusions The identification of a wide range of co-infecting viruses, particularly multiple HPV genotypes, highlights the complexity of viral interactions and their potential implications for public health. These findings enhance our understanding of viral co-infections and provide valuable insights for public health interventions in Sierra Leone. Further research is needed to explore the clinical significance of these findings and their impact on disease outcomes.

Keywords Metagenomics, Virome, SARS-CoV-2, HPV infection, Sierra Leone

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Background

The emergence of the COVID-19 pandemic has significantly tested global capabilities for disease prevention and control. Viral genome sequencing has been instrumental in managing outbreaks and informing public health strategies; however, its implementation is often obstructed in low-resource settings by logistical, financial, and technical constraints [1]. In remote regions, these challenges are exacerbated, limiting the widespread adoption of sequencing for disease monitoring and control [2]. In Sierra Leone, as in many low and middle-income countries (LMIC), these barriers necessitate innovative approaches to enhance the understanding of viral co-infections and their impact on public health [3].

To address these hurdles, our study employed a retrospective metagenomic analysis of SARS-CoV-2 positive nasopharyngeal swab specimen from Sierra Leone. This approach was chosen to gain insights into the spectrum of viruses that may co-infect with SARS-CoV-2, potentially complicating diagnostics, treatment, and disease management [4, 5]. Additionally, by leveraging metagenomic technology, we aimed to assess its viability as a tool for public health surveillance in settings with limited resources. The lessons learned from this study could inform the development of strategies to improve disease control and prevention in Sierra Leone and other LMIC facing similar challenges.

The importance of metagenomic sequencing in public health is increasingly recognized, particularly in the context of infectious diseases [6]. Metagenomic studies have been instrumental in identifying novel viruses and understanding viral diversity in human populations, which is crucial for developing effective vaccines and therapeutics [7]. Applying this technology in LMIC holds promise for enhancing disease surveillance and response, provided that the challenges of accessibility and resource limitations are adequately addressed.

Methods

Sample collection and processing in local laboratory

The workflow consists of three main parts: fieldwork in Sierra Leone, sample transfer, and sequencing in China. A total of 98 SARS-CoV-2 positive nasopharyngeal swab samples were included in this study. Individuals diagnosed between February 2021 to February 2023 underwent SARS-CoV-2 nucleic acid testing at local facilities. The remaining swabs were stored at −40 °C until the commencement of this study. The total nucleic acid was extracted from the samples (Bioperfectus, China) and performed confirmation testing for SARS-CoV-2 using RT-qPCR (Bioperfectus, China). Subsequently, reverse transcription reaction (ThermoFisher, USA) and multiplex displacement amplification (Qiagen, Germany) were conducted. The amplified products were pipetted onto FTA Elute cards (Qiagen, Germany) and transported to the laboratory of China CDC at room temperature under dry conditions for further processing.

Metagenomic sequencing and virome analysis

Nucleic acids were eluted from the FTA cards using QIAcard FTA Elute Buffer (Qiagen, Germany). Nucleic acid samples passing quality control were constructed libraries and sequenced on the Novaseq platform (Illumina, USA) with 150 bp length and paired-end sequencing. To control for potential environmental contamination, the blank FTA cards were subjected to synchronized processing. Sequencing data analysis was completed using our in-house metagenomic analysis pipeline [8]. Briefly, quality control was conducted using fastp (v0.23.4). Next, the data was aligned to the human reference genome using Bowtie2 (v2.3.5.1) to filter out human genome sequences. Subsequently, reads belonging to known cellular organisms were excluded using diamond (v2.1.8). For virus identification, the remaining reads were matched against viral nucleotide and protein databases using blastn (v2.14.0) and diamond, respectively. Taxonomies with aligned reads showing optimal BLAST scores were resolved using the MEGAN6 Metagenome Analyzer (v. 6.24.23).

HPV identification and phylogenetic analyses

To classify HPV more accurately, the datasets were aligned with the HPV reference genomes (PAVE, https://pave.niaid.nih.gov/) separately [9, 10]. All aligned reads were obtained and conducted de novo assembly on the data using megahit software (v1.2.9). Then, the assembled overlapping fragments were compared and subjected to phylogenetic analysis using the reference genome and HPV L1 gene sequence (IHRC, https://www.hpvcenter.se/) to determine the genotype of HPV in each sample individually [11, 12]. Furthermore, all near full-length genomes, defined as those exceeding 60% of the length (4800 base pairs) of the reference genomes, were submitted to Geneious primer (v2024.0.4) for structural gene prediction. The chi-square test was utilized to examine differences in HPV positive rates among different genders, age groups, and residential locations, using GraphPad software (v.9.0.0), with a significance level set at 0.05.

Results

Sample collection and socio-demographic characteristics

All 98 infected individuals in this study were residents of Sierra Leone for over 6 months. They were undergoing SARS-CoV-2 testing to apply for international travel documents. Out of the samples, 47 were from males, 41 were from females, and gender information was missing

for 10 samples. Meanwhile, 67 were adults aged 18–59, 2 were infants under the age of 1, and 4 were elderly individuals aged 60 and above. Based on the residence of the infected individuals, 84 lived in Freetown, 2 lived in other areas, and 12 were unknown (Table 1).

Virus spectrum identification

A total of 3569 million reads were obtained from 98 samples. Among them, the mean data size of the samples was 5.4 G data (Supplementary Table 1). Totally 22 viral taxa were identified in all samples, which belongs to 20 viral families (Fig. 1), including 4 human viruses (Coronaviridae, Anelloviridae, Papillomaviridae and Polyomaviridae), 2 fungi virus (Totiviridae and Partitiviridae), 3 other virus (Bunyavirales, Ciroviridae and Genomoviridae) and 11 viral families of Bacteriophage. SARS-CoV-2 were detected in 74 out of 98 samples, with the relative abundance varied. Upon retesting the samples that did not initially detect SARS-CoV-2 using RT-qPCR, high ct values were observed. This could be due to viral RNA degradation during long-term storage. The data is deposited in National Microbiology Data Center (NMDC) with Accession Numbers NMDC10018701 (https://nmdc.cn/resource/zh/genomics/project/detail/NMDC10018701).

Table 1 Socio-demographic characteristics of 98 SARS-CoV-2 positive individuals

	Gender			Total
	Female	Male	Unknown	
Collection time				
2021	21	22	4	47
2022	20	24	6	50
2023	–	1	–	1
Age group				
< = 1	–	2	–	2
2–17	2	3	2	7
18–39	18	19	3	40
39–59	9	17	1	27
> = 60	2	2	–	4
Unknown	10	4	4	18
Collection district				
Western Area Urban	32	39	9	80
Western Area Rural	8	6	1	15
Moyamba	1	–	–	1
North West Province	–	1	–	1
Port Loko	–	1	–	1
Collection site				
Community	28	32	8	68
Hospital	13	15	2	30
Residence place				
Freetown	37	38	9	84
Kaffubullom	–	1	–	1
Kaiyamba	1	–	–	1
Unknown	3	8	1	12
Total	41	47	10	98

HPV identification and phylogenetic analyses

In 19 individuals, human papillomavirus (HPV) was identified, and metagenomic analysis revealed the presence of various genotypes. Totally 34 different HPV types were identified, which belong to beta (n = 22) and gamma (n = 12) Papillomavirus, but high-risk types such as HPV-16 or HPV-18 were not found (Supplementary Table 2). Most putative HPV genomes or L1 gene Identity to classified or unclassified HPV genome > 90%, defined as new variant or sub-type of HPVs (Fig. 2A, B) [12, 13]. Furthermore, structural gene prediction revealed that these putative genomes encode 6 or 7 major structural proteins, which exhibit a high degree of consistency with the proteins encoded by betapapillomavirus and gammapapillomavirus, respectively (Fig. 2C). The putative genomes reported in this study were deposited in the GenBank with accession numbers PP296644–PP296687.

HPV infection rate and multiple infection

The HPV positive rate was 19.5% (8/41) in females and 21.3% (10/47) in males, with no significant difference. Although 20% (16/80) of HPV-positive cases resided in urban, higher than 6.7% (1/15) in rural, the difference was not significant. Similarly, there was no significant difference in age groups, which may be related to the small sample size. Multiple HPV infections were observed in 8 individuals, with 2–13 different types (subtypes or variant) of HPV identified in one case (Table 2). The highest number of HPV types was found in a 1-year-old boy, who had 4 types of beta- and 9 types of gamma- HPVs detected in his sample (Supplementary Table 2). The second highest number of HPV types was found in a 23-year-old male, who was infected with eight types of HPV.

Discussion

In nature, co-infection with viruses is as widespread as single-virus infections. Co-infection typically leads to alterations in viral pathogenic, disruption of host defenses, and confusion of clinical symptoms, all of which contribute to making the diagnosis and treatment of the disease more challenging [4, 14]. In this study, retrospective metagenomic study was conducted to investigate the spectrum of viral co-infections in SARS-CoV-2 positive individuals in Sierra Leone. Over 20 viral taxa co-infecting with SARS-CoV-2 were discovered. In our study, apart from SARS-CoV-2 and HPV, we identified human viruses belonging to the Anelloviridae family,

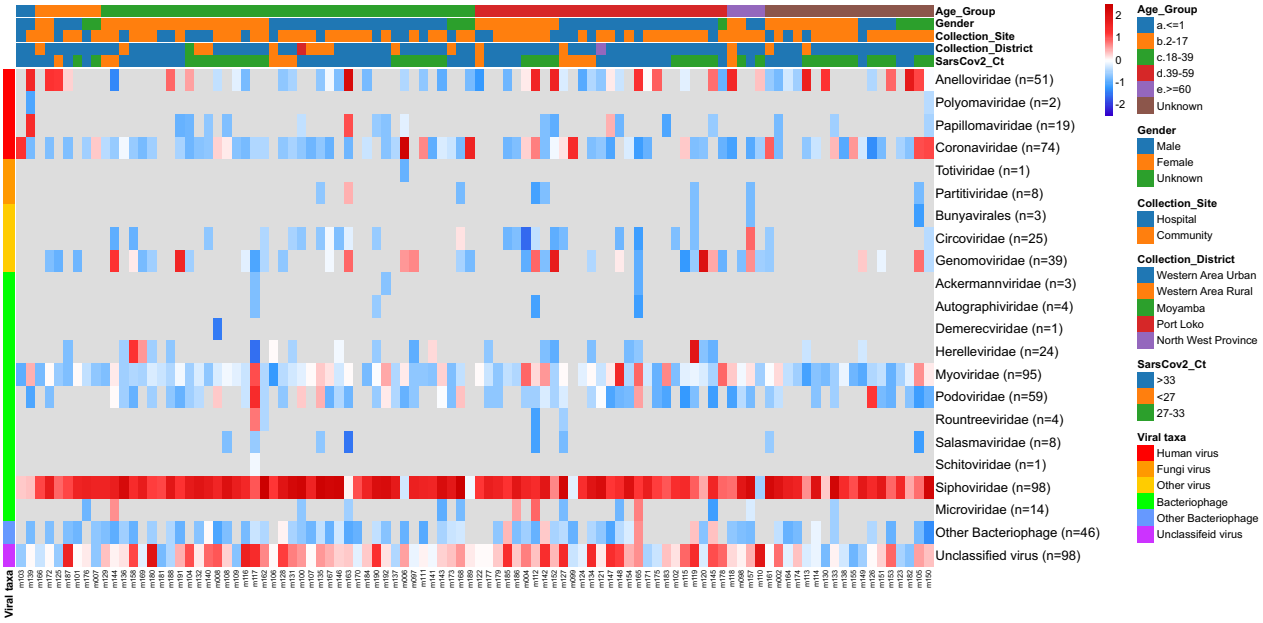

Fig. 1 Heat-map revealed the virome identified in 98 SARS-CoV-2 positive nasopharyngeal swab samples. Each column in the figure represents a sample The color bar on the left displays the classification of viruses, and the color bar at the top shows the basic characteristics of the cases. The color of the blocks represents the relative abundance of the virus, which has been normalized by each sample for data processing. Red represents high relative abundance, and blue represents low relative abundance

specifically the genera Alphatorquevirus (n = 5), Betatorquevirus (n = 7), and Gammatorquevirus (n = 6), with a total of 51 individuals testing positive [15]. It is worth noting that while the Polyomaviridae family has a broad host range, including mammals, birds, and fish, we report the presence of Alphapolyomavirus (n = 1) in humans. The sequences in other samples were fragmented, making it difficult to determine specific species or genera. Despite the widespread prevalence of Anelloviruses in the population, their association with human diseases is not well established [16]. Similarly, most human infections with Polyomaviruses seem to be asymptomatic or cause minimal symptoms [17]. Furthermore, there are two types of fungi viruses, as well as three other viruses that affect plants, insects, birds, or other mammals. It is possible that these viruses are related to various factors such as the environment and diet. The results suggest a rich diversity of viral communities in the local population. However, as the nasopharyngeal region is connected to both the nasal and oral cavities, samples are susceptible to contamination from the external environment. Therefore, the relationship between viruses discovered through metagenomics in the nasopharyngeal and diseases, as well as the potential existence of emerging or re-emerging infectious diseases, warrants further investigation.

HPV commonly infects the population and is a significant contributing factor to various cancers, thus receiving attention [18, 19]. Among these, cervical cancer's incidence is steadily rising in the sub-Saharan African region, imposing an exceptionally heavy disease burden [20, 21]. In this study, we observed a prevalence of 19.4% (19/98) HPV infections in the nasopharyngeal among the local population, with the presence of multiple infections involving various variants or subtypes. Limited resources in Sierra Leone have hindered the widespread implementation of HPV infection screening, despite efforts to address the issue [22]. Our observations on HPV types, specifically HPV-22 and HPV-80, differ from previous studies conducted on populations in West Africa [23]. This variation can be attributed to differences in HPV distribution among various countries and populations, as well as the fact that earlier research primarily focused on cervical cancer in females, with limited investigation into oral HPV infection in West Africa region [21].

Available evidence suggests that transmission of oral HPV infection is likely to occurs through oral sexual contact or vertically from mother to child during childbirth [24]. Studies also indicate potential links between nasopharyngeal HPV infections and oropharyngeal cancer occurrence [25, 26]. Such associations are especially concerning in children, aged or immunocompromised people, as even non-high-risk HPV can impact immune status and trigger diseases [27, 28]. However, as a retrospective study, we lack clinical data to conclusively determine if these HPV infections correlate with diseases.

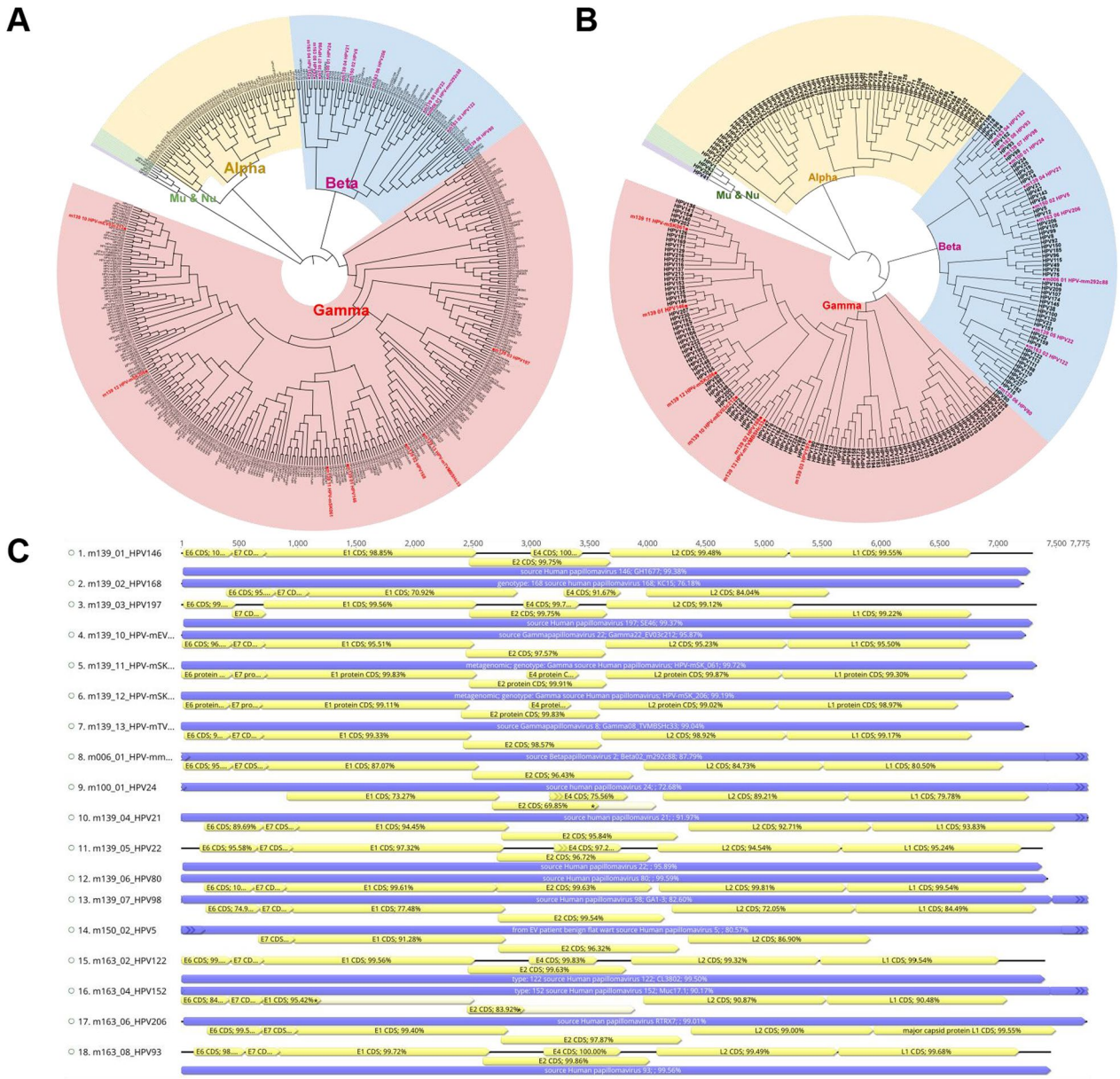

Fig. 2 Phylogenetic analysis and ORF prediction of putative HPV genomes identified in nasopharyngeal swab samples, Sierra Leone. The phylogenetic trees constructed for putative Human Papillomavirus (HPV) genomes (**A**) and L1 gene sequences (**B**), respectively, highlighting sequences discovered in this study (marked in red for Gamma HPV and purple for Beta HPV). The open reading frame (ORF) prediction for identified betapapillomavirus and gammapapillomavirus genomes (**C**)

Overall, the relatively high nasopharyngeal HPV prevalence may constitute a public health issue in Sierra Leone. Further research should evaluate correlations with cancers like oropharyngeal cancer.

Conclusion

The findings of this retrospective metagenomic study in Sierra Leone reveal a significant burden of HPV co-infections among SARS-CoV-2 positive individuals, with 19.4% of samples showing HPV presence and a diverse range of 34 HPV types identified. The predominance of beta and gamma Papillomavirus genera and the occurrence of multiple HPV types within single individuals underscore the complexity of viral interactions and their potential public health implications, particularly concerning cancer risks. The study not only highlights the utility of metagenomic sequencing for disease surveillance in low-income settings but also underscores the

Table 2 HPV identified in 19 nasopharyngeal swab samples and their characteristics

ID	HPV type[a]	HPV unclassified[a]	Multiple infection	HPV genotype	Age	Gender	Collection district	Residence place
m002	HPV-49		1	Betapapillomavirus	Unknown	Female	Western Area Urban	Unknown
m006		HPV-mm292c88nr	1	Betapapillomavirus	34	Male	Western Area Urban	Freetown
m100	HPV-24	HPV-mSK043nr	2	Betapapillomavirus	39	Male	Port Loko	Kaffubullom
m104	HPV-47		1	Betapapillomavirus	31	Female	Moyamba	Kaiyamba
m108	HPV-188/195		2	Beta & Gamma	22	Female	Western Area Urban	Freetown
m133	HPV-14		1	Betapapillomavirus	Unknown	Female	Western Area Urban	Freetown
m139	HPV-21/22/80/98/146/168/197	HPV-mdo1c232nr/mEV03c05nr/mEV03c212nr/mSK061nr/mSK206nr/mTVMBSHc33nr	13	Beta & Gamma	1	Male	Western Area Urban	Freetown
m140	HPV-47		1	Betapapillomavirus	29	Female	Western Area Rural	Freetown
m142		HPV-mFS1nr	1	Gammapapillomavirus	51	Female	Western Area Urban	Freetown
m145	HPV-21	HPV-mm292c88nr	2	Betapapillomavirus	52	Male	Western Area Urban	Unknown
m147	HPV-22/80/197	HPV-mEV03c212nr/mSK061nr/mSK206nr	6	Beta & Gamma	54	Male	Western Area Urban	Freetown
m148	HPV-22		1	Betapapillomavirus	46	Male	Western Area Urban	Unknown
m150	HPV-5/75/174		3	Betapapillomavirus	Unknown	Unknown	Western Area Urban	Freetown
m152	HPV-5/80		2	Betapapillomavirus	41	Female	Western Area Urban	Freetown
m163	HPV-14/93/115/122/152/196/206/222		8	Beta & Gamma	23	Male	Western Area Urban	Freetown
m183		HPV-mHIVGc36nr	1	Betapapillomavirus	44	Male	Western Area Urban	Unknown
m190	HPV-182		1	Betapapillomavirus	37	Male	Western Area Urban	Freetown
m191		HPV-mEV03c212nr	1	Gammapapillomavirus	32	Female	Western Area Urban	Freetown
m192	HPV-174		1	Betapapillomavirus	33	Male	Western Area Urban	Freetown

[a] Most putative genomes or L1 gene identity to classified or unclassified HPV genome > 90%, defined as new variant or subtypes of HPVs. The HPV type is represented in the table by the closest identity classified or unclassified HPV genome

necessity for further research to elucidate the clinical significance and impact of these co-infections on disease outcomes. This knowledge is critical for informing public health strategies, including HPV screening and vaccination programs, ultimately aiming to alleviate the burden of HPV-related diseases in Sierra Leone and similar contexts.

Abbreviations:

SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
COVID-19	Coronavirus Disease 2019
LMIC	Low- and Middle-Income Countries
HPV	Human papillomavirus
FTA	Flinders Technology Associates
RT-qPCR	Real-time quantitative reverse transcription PCR

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12985-024-02466-z.

Supplementary Material 1: Table 1. Summary of Sequencing Data and Processing Outcomes. The table provides a summary of the key data and outcomes from the sequencing process, which includes the raw reads, clean reads, non-human mapped reads, human genome percentage, and viral reads for each sample.

Supplementary Material 2: Table 2. All HPVs identified in 19 SARS-CoV-2 positive nasopharyngeal swab samples. The putative HPV type is represented in the table by the closest identity classified or unclassified HPV genome.

Author contributions

Conceived and designed the experiments: X.H., X.M., D. H. and X.D. Sample collection: X.H., A.T., Q.Y., L.G., L.W. and T.T. Performed the experiments: X.H., A.T., Y.Z. and F.T. Analyzed the data: X.H., L.W., K.X. and F.T. Contributed reagents/materials/analysis tools: Q.Y., W.W. and X.M. Wrote the paper: X.H., A.T., D.H. and X.D. All authors have read and agreed to the published version of the manuscript.

Funding

This study was supported by National Key Research and Development Program (Grant No. 2020YFE0205700) and Capital's Funds for Health Improvement and Research (Grant No. 2022-1G-1131).

Availability of data and materials

The metagenomic sequencing data is deposited in National Microbiology Data Center (NMDC) with accession numbers NMDC10018701. The putative HPV genomes reported in this study were deposited in the GenBank with accession numbers PP296644–PP296687.

Declarations

Ethical approval and consent to participate

The sampling and experimental procedures of this study were reviewed by the institutional review board of China CDC (Approval Notice No. 202113) and Office of the Sierra Leone Ethics and Scientific Review Committee (1.0 of September, 2021).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Received: 11 June 2024 Accepted: 12 August 2024
Published online: 27 August 2024

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