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Review

Characterization of human respiratory syncytial virus in children with severe acute respiratory infection before and during the COVID-19 pandemic

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ABSTRACT

Objectives: Annual outbreaks of human respiratory syncytial virus (HRSV) are caused by newly introduced and locally persistent strains. During the COVID-19 pandemic, global and local circulation of HRSV significantly decreased. This study was conducted to characterize HRSV in 2018-2022 and to analyze the impact of COVID-19 on the evolution of HRSV.

Design/methods: Combined oropharyngeal and nasopharyngeal swabs were collected from children hospitalized with severe acute respiratory infection at two hospitals in Zambia. The second hypervariable region of the attachment gene G was targeted for phylogenetic analysis.

Results: Of 3113 specimens, 504 (16.2%) were positive for HRSV, of which 131 (26.0%) and 66 (13.1%) were identified as HRSVA and HRSVB, respectively. In early 2021, an increase in HRSV was detected, caused by multiple distinct clades of HRSVA and HRSVB. Some were newly introduced, whereas others resulted from local persistence.

Conclusions: This study provides insights into the evolution of HRSV, driven by global and local circulation. The COVID-19 pandemic had a temporal impact on the evolution pattern of HRSV. Understanding the evolution of HRSV is vital for developing strategies for its control.

Introduction

The molecular evolution of human respiratory syncytial virus (HRSV) can be associated with antigenic diversity that affects epidemiological patterns [1]. Therefore, understanding the evolution of HRSV is crucial for establishing effective prevention strategies, such as vaccines. Globally, HRSV is the leading viral agent responsible for acute lower respiratory infections [2]. An analysis of the disease burden in children revealed overall deaths between (84,500-125,200) in children aged 0-60 months, most of which occurred in low- and middle-income countries [3].

HRSV is an enveloped virus with a single-stranded nonsegmented negative-sense RNA. HRSV is a member of the *Pneumoviridae* family, belonging to the genus *Orthopneumovirus* [4]. The genome is 15-kb long with 10 gene transcripts encoding 11 proteins, of which attachment glycoprotein G (G gene) and fusion glycoprotein F (F gene) are major surface proteins. G and F genes function in viral attachment and penetration by fusion between the virion envelope and host cell plasma membrane [5,6]. HRSV has a single serotype with two major subgroups (A and B) based on reactions with monoclonal antibodies and genetic variability of G and F genes [5,6]. According to the newly proposed classification of Goya, HRSVA is classified into GA1-GA3 genotypes [7]. The predominant HRSVA genotype in circulation is ON1 (now GA2.3.5), which emerged in 2011 and contains a 72-nucleotide duplication in the second hypervariable region of the G gene [8]. HRSVB has been reclassified into GB1-GB7 genotypes [7]. The predominant genotype BA9 (now

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GB5.0.5a) is characterized by a 60-nucleotide duplication within the second hypervariable region of the G gene [9]. The second hypervariable region contains strain-specific amino acid substitutions in epitopes that are frequently sequenced to ascertain genotype (lineage or clade) [10]. G gene is predominantly impacted by N-linked glycosylation, which is used for host immune evasion and host attachment. The sugar moieties of G protein conceal antigenic regions, enabling immune evasion [11].

During the early stage of the COVID-19 pandemic, reductions in respiratory virus infections, including those caused by HRSV, were noted [12]. These reductions were a result of mitigation measures, such as border control and physical distancing measures, including lockdowns to prevent the spread of the COVID-19 pandemic [2]. HRSV cases have increased as control measures have been gradually lifted, possibly because of increased international traffic and social contact in populations with declining immunity [13].

The annual epidemics of HRSV are caused by newly introduced and local persistence strains [14]. A broad genetic pool is essential for maintaining the transmission of respiratory viruses, such as influenza virus and HRSV. The sudden decline in infections could have led to a genetic bottleneck that lowered viral diversity [12].

To the best of our knowledge, there is no previous study in Zambia on the evolution of HRSV before and during the COVID-19 pandemic. Therefore, continuous monitoring of circulating HRSV strains is necessary to monitor the evolution patterns before and during the COVID-19 pandemic. In this study, we aimed to determine the molecular characteristics of HRSVA and HRSVB in children with severe acute respiratory infection (SARI) from 2018 to 2022 in Zambia.

Materials and methods for respiratory syncytial virus detection

Study period and location

The study participants were enrolled between January 2018 and December 2022 from two sentinel surveillance sites in Lusaka and Ndola—two referral hospitals: The University Teaching Hospital Children's Hospital, Lusaka, Lusaka Province and Arthur Davison Children Hospital (ADH), Ndola, Copperbelt Province. Lusaka—the capital of Zambia—has an area of 21,896 km^2 and a population of >3.6 million people. Ndola is the administrative town of the Copperbelt Province and the third-largest city in Zambia. Ndola has a population of approximately 620,000 people.

Study design and specimen collection

A revised case investigation form (CIF), proposed by the World Health Organization, was used in this study [15]. SARI was defined as presenting with manifestations of acute respiratory infection with a temperature ≥38°C, history of fever and cough, onset within the last 10 days, and requiring hospitalization. This study enrolled only children below the age of 5 years. A combined oropharyngeal and nasopharyngeal swab was collected from the children. Specimens were taken as soon as possible for any case(s) that meet the sampling criteria, defined according to the SARI surveillance protocol. The specimens were immediately labelled, refrigerated (4°C) in a viral transport medium (Copan, Brescia, Italy) and sent to the sentinel site laboratory or other designated holding area with the CIF. Finally, the specimens were transported to the National Influenza Center (NIC) at the Virology Laboratory, University Teaching Hospital, Lusaka for storage at −80°C and subsequent processing. Patent information and clinical details collected using the CIF were entered into the Access database.

RNA extraction and complementary DNA synthesis

RNA was extracted from the collected specimens using a QIAmp mini extraction kit (Qiagen, Hamburg, German), following the manufacturer's instructions. The specimens were tested for influenza A virus, influenza B virus, and SARS-CoV-2 using Centers for Disease Control and Prevention Influenza SARS-CoV-2 (Flu SC2) Multiplex Assay (Catalog No. FluSC2PP-RUO) (Atlanta, GA, USA). Specimens negative for influenza A and B and SARS-CoV-2 were tested for HRSV. A specimen with a ribonucleic protein gene cycle ≤37 was considered valid for HRSV testing. Complementary DNA was synthesized using Moloney Murine Leukemia Virus reverse transcriptase and random primers (Invitrogen, Carlsbad, CA, USA).

Data analysis

SPSS statistics version 25 (IBM Corp., Armonk, NY, USA) was used to compute the proportion of HRSV-positive cases.

Molecular screening and sequencing of human respiratory syncytial virus

Real-time polymerase chain reaction (PCR) was used to screen HRSV, targeting the nucleocapsid gene, with an adopted protocol [16] using forward primer: GCTCTTAGCAAAGTCAAGTTRAATGATACA, reverse primer: GTTTYTGCACATCATAATTRGGAGT, and TaqMan probe: (5′-VIC) CTRTCATCCAGCAAATA-(MGB-3′). HRSV was further classified into HRSVA or HRSVB by partial sequencing targeting the C terminal region of HRSV G. As described [17,18], for HRSVA, two sets of forward primers were used for the first and nested PCRs (5′-GAAGTGTTCAACTTTGTACC-3′ and 5′-TATGCAGCAACAATCCAA-3′). A common reverse primer was used for the first and nested PCRs (5′-CAACTCCATTGTTATTTGCC-3′). For HRSVB, two forward primers were used (5′-AAGATGATTACCATTTTGAAGT-3′ and 5′-GTGGCAACAATCAACTCTGC-3′) for nested PCR. A common reverse primer was used for first and nested PCRs (5′-CAACTCCATTGTTATTTGCC-3′). The PCR products from the first PCR or nested PCR were purified from agarose gels using Illustra ExoProStar (Global Life Sciences Solutions, MA, USA). This was followed by cycle sequencing in the forward and reverse directions with primers of the first PCR or nested PCR using Big Dye Terminator v3.1 cycle sequencing kit and Genetic Analyzer 3730 (Applied Biosystems, Foster City, CA, USA).

Sequence alignment and phylogenetic construction

The HRSVA and HRSVB sequences were examined and edited using BioEdit (Version 7.1.3). Consensus sequences were obtained using Geneious Prime (Version 2023.1.2). The sequences were deposited in GenBank with the following accessions: HRSVA (OR466207–OR466323) and HRSVB (OR455893–OR455951). Multiple sequence alignment was performed in MAFFT (Version 7.0). The best-fit substitution model identified for tree construction was the Tamura-Nei (TN93 + G) model using the IQ-TREE ModelFinder [19]. Maximum likelihood analysis was performed for HRSVA and HRSVB in MEGA (Version 7.0); the reliability of the branching order was estimated by performing 1000 bootstrap replicates. To identify the source of the Zambian sequences, we included sequences from other countries obtained from GenBank and Global Initiative on Sharing All Influenza Data and performed the maximum likelihood phylogenetic analysis using TimeTree [20] and FigTree (Version 1.4.4). Pairwise distance (p-distance) between clades was calculated in MEGA (Version 7.0) and used to determine the HRSV genotypes. Our study focused on the second hypervariable region of G protein because only alterations in this region may bring about introductions or persistence of strains. Therefore, strains with sequences containing less than four nucleotide differences in the G gene from a variant detected in the immediate one or two preceding epidemics were considered persistent variants; according to Agoti, most of these less than four nucleotide changes occurred in the second region of the G gene [21].

Table 1
Detection of HRSV in hospitalized children with severe acute respiratory from 2018 to 2022 in Zambia.

	Lusaka				Ndola			
	HRSVA	HRSVB	Untypable	Total	HRSVA	HRSVB	Untypable	Total
Year								
2018	24 (3.4)	0 (0.0)	40 (5.6)	715	32 (32.7)	0 (0.0)	8 (8.2)	98
2019	11 (3.5)	1 (0.3)	41 (12.9)	318	8 (2.8)	0 (0.0)	38 (13.2)	288
2020	2 (0.9)	19 (8.5)	12 (5.4)	224	0 (0.0)	0 (0.0)	0 (0.0)	0
2021	4 (0.9)	43 (10.1)	58 (13.6)	425	9 (3.9)	3 (1.3)	35 (22.4)	228
2022	35 (7.8)	0 (0.0)	38 (8.4)	451	6 (1.6)	0 (0.0)	37 (10.1)	366
Total	76 (3.6)	63 (3.0)	189 (8.9)	2133	55 (5.6)	3 (0.3)	118 (12.0)	980

HRSV, human respiratory syncytial virus.
The numbers in parentheses indicate percentages.

Alignment of deduced amino acids and identification of N-glycosylation sites

Amino acid mutations were deduced by translating with the standard genetic code in MEGA (Version 7.0). N-glycosylation sites were computed using the NetNglyc server, which predicts N-glycosylation sites in human proteins using artificial neural networks that examine the sequence context of Asn–Xaa–Ser/Thr sequons [22].

Analysis of selection pressure

Sequences of partial G gene from HRSVA and HRSVB were tested for evidence of natural selection using the web-based Datamonkey 2.0 tool. Fast Unconstrained Bayesian Approximation (FUBAR) was used for proof of pervasive selection, whereas the mixed-effects model of evolution (MEME) was used to test for proof of episodic selection [23]. Synonymous and non-synonymous substitution rates were determined using MEGA (Version 7.0) and HyPhy software package under the Muse–Gaut model of codon substitution and Felsenstein's 1981 model of nucleotide substitution. MEME and HyPhy used a statistical significance threshold of 0.5. FUBAR produced outcomes characterized by a strong posterior probability of 0.9, indicating a high chance of frequent transience.

Results

HRSV detection

Overall, 3,113 children with SARI were enrolled in this study between January 2018 and December 2022. In Zambia, the first COVID-19 cases were identified in March 2020 and the first mitigation measures were implemented in the same month. SARI specimen collection was temporarily stopped between May and July 2020 because of COVID-19. The cases enrolled from 2018 to April 2020 and from July 2020 to 2022 were considered cases enrolled before and during the pandemic, respectively. In total, 504 (16.2%) HRSV cases were detected, of which 131 (26.0%) were HRSVA, 66 (13.1%) were HRSVB, and 307 (60.9%) were untypable (Table 1).

Seasonal patterns of HRSV in Zambia

In Zambia, the weather pattern is divided into three categories as follows: dry-cool (between May and mid-August), dry-hot (between August and November), and warm-wet (between mid-November and April). A total of 306 HRSV cases were seen during the warm-wet period, 169 of the cases were seen during the dry-cool period, and 29 during the dry-hot period (Figure 1 and Supplementary Figures 1 and 2).

Phylogenetic analysis of the partial HRSV G gene among strains isolated in 2018-2022 in Zambia

Phylogenetic trees were constructed for HRSVA and HRSVB (Figure 2). Only 131 were sequenced successfully and all clustered within genotype ON1, in which five distinct clades were observed. Clades 1 and 2 were only observed before the pandemic. Clade 1 constituted cases from 2018 to 2019, whereas clade 2 constituted cases from 2018. Most sequences in these clades are homogenous, and a Basic Local Alignment Search Tool search could not identify similar sequences in other countries (data not shown). Strains in Clade 1 with homologous sequences were considered persistent because the sequences were identified in 2018 and 2019.

Clades 3 and 5 of HRSVA showed different patterns from Clades 1 and 2. The strains in Clades 3 and 5 were detected in several years, with high diversity. Two small clusters from Clade 5 were considered persistent: one from 2018 to 2019 and another from 2019 to 2021. Clade 3 lacked persistent strains. Unlike that for Clades 1 and 2, similar sequences were identified in other countries from different regions.

All HRSVB sequences (n = 66) were clustered within the genotype BA9, for which four clades were detected. Most strains were classified into Clades 1 and 4, with high diversity. However, similar sequences in Clade 1 were identified in other countries and no persistent strain was identified in this clade. In Clade 4, a Basic Local Alignment Search Tool search could not identify similar sequences in other countries (data not shown). Many sequences in Clade 4 were grouped in a cluster with homogenous strains. These strains were identified in 2020 and 2021 and thus were considered persistent. Notably, these strains were considered persistent from the pre-COVID-19 pandemic in 2020 to the COVID-19 pandemic in 2021. A small number of sequences were identified for Clades 2 and 4 without any persistent strain.

The HRSVA ON1 and HRSVB BA9 clades identified in this study clustered with other sequences worldwide. Sequences of HRSVA from Oceania, North America, and Europe showed distinct clustering, indicating possible local evolution within the region. The uniquely Zambian clades (Clades 1 and 2) of HRSVA ON1 clustered only with the Zambia strain in global phylogeny (Supplementary Figure 3). Unlike HRSVA, HRSVB strains from different regions clustered together, suggesting more frequent global dissemination of the BA9 genotype. Clade 4, unique to Zambia, is distinctively seen by branching with only Zambian strains (Supplementary Figure 4).

Calculation of average p-distance between clades in SARI cases

We calculated the average p-distance between the HRSVA clades deduced from this study and the HRSVA ON1 prototype strain. The average p-distance was 0.04, whereas the average p-distance between the HRSVB clades deduced from this study and the HRSVB BA9 prototype strain was 0.05. We determined the p-distance within each year of the study, followed by p-distance between seasons. Next, the p-distance between 2019 versus 2020 and 2020 versus 2021 for HRSV A was relatively high at 0.047, whereas HRSVB strains in 2021 showed high divergence, with p-distance = 0.048. In addition, the p-distance between these two seasons for 2020 versus 2021 was 0.051 (Table 2).

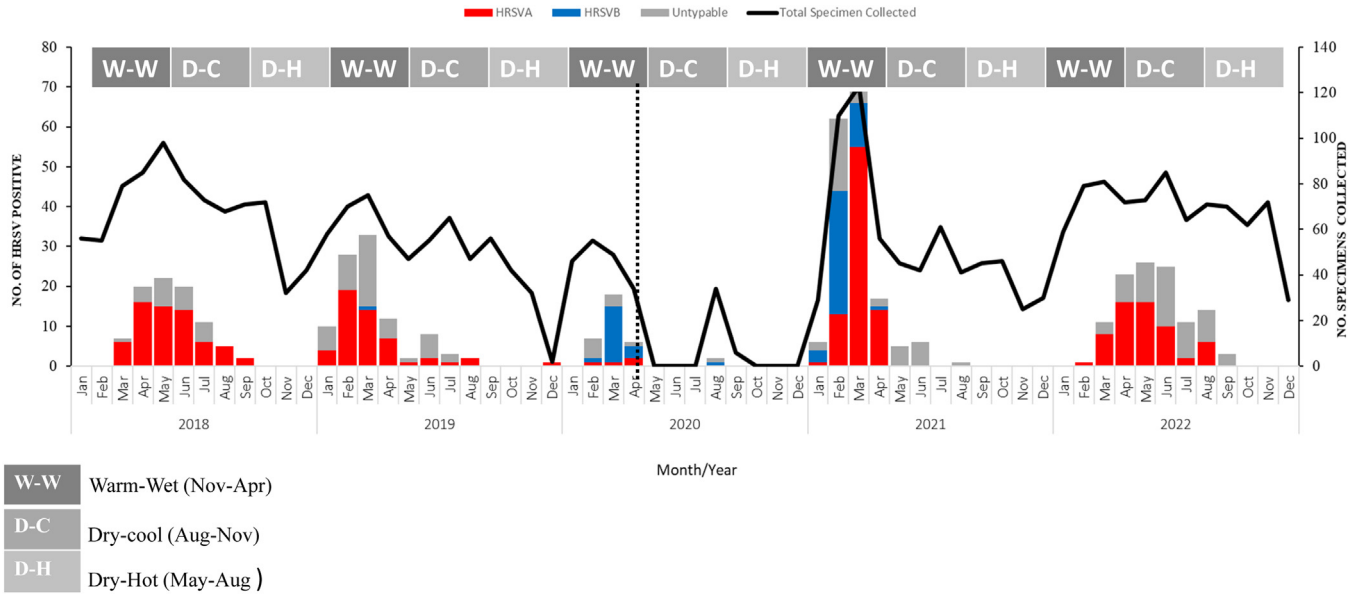

Figure 1. Monthly distribution of HRSV cases detected in 2018-2022 in Zambia. The number of specimens collected by month is shown with a black line (right scale). HRSV-positive cases are indicated by stack bars. HRSVA and HRSVB cases are shown with red and blue bars, respectively. Untypable HRSV is indicated with grey bars. The dotted line indicates the start of the COVID-19 pandemic in Zambia. HRSV, human respiratory syncytial virus.

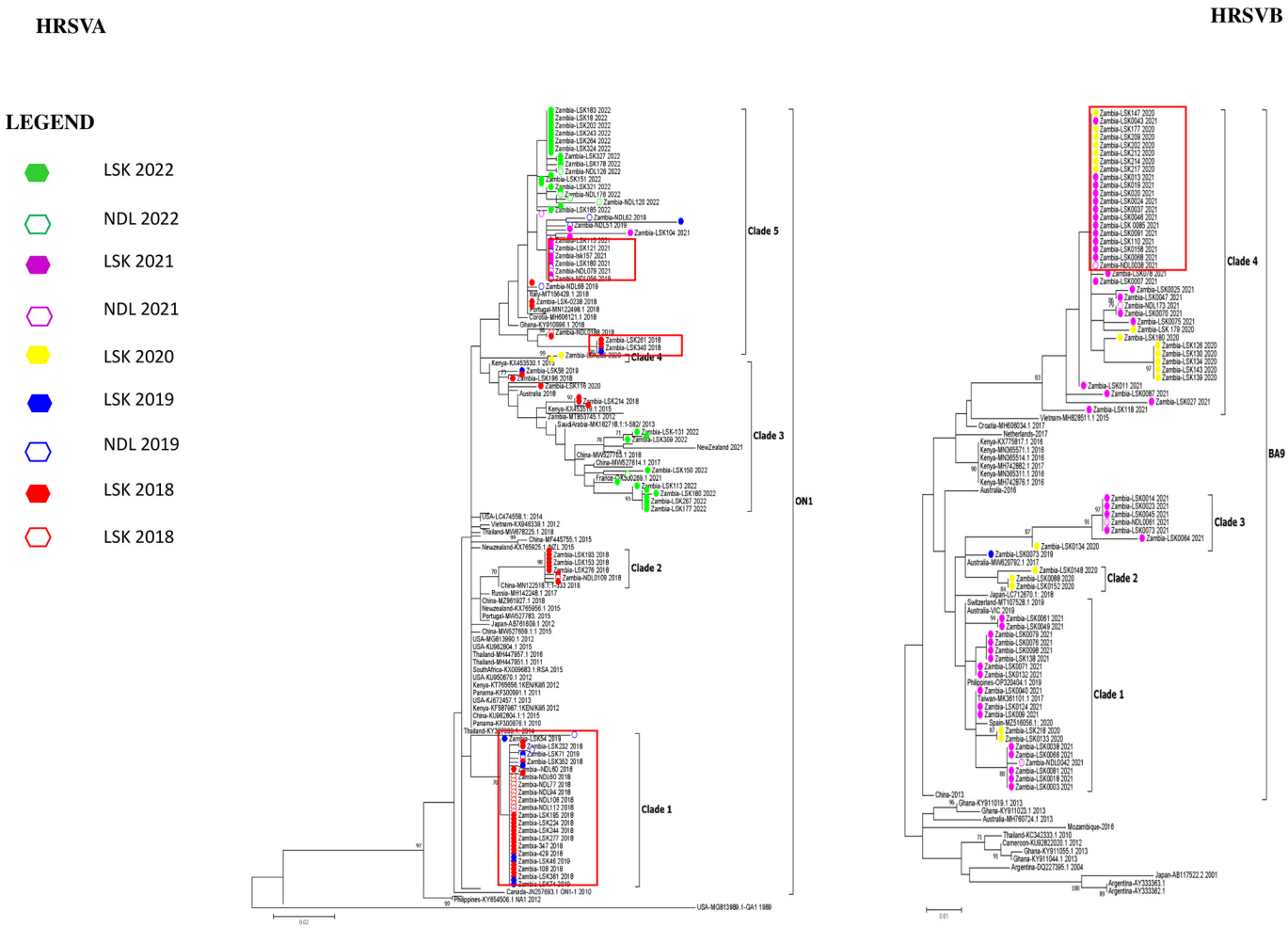

Figure 2. Phylogenetic tree of the second hypervariable region of HRSVA (left) and HRSVB (right) genotypes circulating in 2018-2022 in Zambia. The trees were inferred using MEGA (Version 7.0) and utilizing the maximum likelihood and bootstrap support of 1000. In this tree, only bootstrap values >70 are shown. Observed distinct Zambian clades from Lusaka (LSK) and Ndola (NDL) are indicated on the left of HRSVA and HRSVB phylogenetic trees. Red squares indicate clades with persistent strains. HRSV, human respiratory syncytial virus.

Table 2
Between and within group average pairwise distance (p-distance) estimation.

Clades		Year		Epidemic Season	
	p-distance between ON1 and BA9 prototypes		p-distance within year		p-distance between seasons
HRSVA ON1					
Clade 1 (40)	0.037	2018	0.032	2018 vs. 2019	0.047
Clade 2 (9)	0.046	2019	0.039	2019 vs. 2020	0.047
Clade 3 (24)	0.051	2020	0.031	2020 vs. 2021	0.047
Clade 4 (2)	NA	2021	0.007	2021 vs. 2022	0.034
Clade 5 (56)	0.058	2022	0.036		
HRSVB BA9					
Clade 1 (19)	0.104	2021	NA		
Clade 2 (3)	0.056	2020	0.034	2020 vs. 2021	0.051
Clade 3 (6)	0.075	2021	0.048		
Clade 4 (38)	0.057				

*Average p-distance among nucleotide sequences between deduced clades and prototype strains and epidemic seasons were calculated by pairwise comparison using the Tamura-Nei model, gamma distributed, including transitions and transversions in MEGA 7.

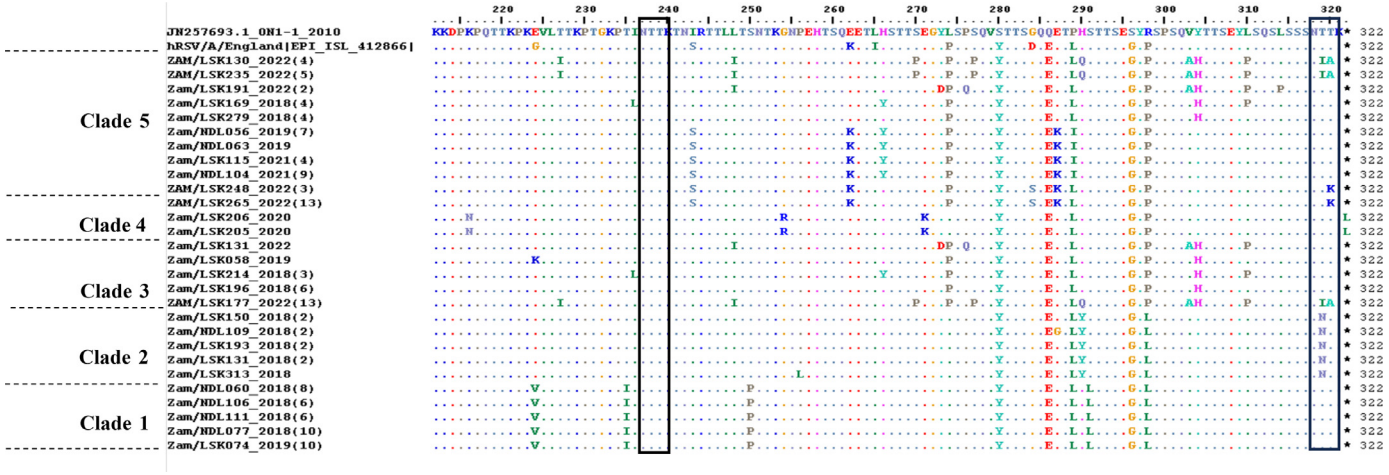

Figure 3a. HRSVA partial G gene amino acid substitutions deduced among SARI cases in 2018-2022 in Zambia. Strains from this study were compared relative to the reference JN257693.1 (ON1 prototype). The number of sequences with additional identical strains is shown in parentheses on the right of the strain name. Dots represent identical residues. Asterisks represent stop codons. Potential N-linked glycosylation is indicated by horizontal black boxes.

Alignment of deduced amino acids for HRSVA and HRSVB and N-linked glycosylation

The changes in amino acids in the six clades were predicated and analyzed using the BioEdit sequence alignment editor (Version 7.2). The sequences were grouped and highlighted based on differences in amino acids (Figures 3 and 4). We identified five clades compared with the reference ON1 genotype (GenBank: accession JN257693.1), two of which (Clades 1 and 2) were predominant in 2018-2019 and are characterized by unique mutations: Clade 2 contained mutations at H290Y and T319N and Clade 1 at E224V, T235I, S250P, and S291L. Two sequences from 2020 (Clade 4) had leucine instead of the stop codon (stop322L).

Of the four HRSVB clades, all strains in Clades 2 and 3 had the stop codon at position 313 replaced by the codon for glutamine (stop313Q), whereas in Clade 4, only 32 strains were replaced.

The analysis of the N-linked glycosylation of the HRSVA reference strain (GenBank: accession JN257693.1) and our strains revealed loss of N-linked glycosylation in 25 strains in clade 5 at position 320 and 13 strains in Clade 3, whereas loss of N-linked glycosylation in HRSVB from our strains compared with the reference strain (GenBank accession DQ227395.1) was observed 19 strains in Clade 1 and 6 strains in Clade 4 and at position 312 (Figures 3a and 3b).

Analysis of selection pressure

For HRSVA, Common positive selection sites were found using FUBAR and MEME methods at 103 and 109. Using MEGA (Version 7.0) and operating the HyPhy software package, we found 17 sites under the positive selection. Although for HRSVB, common positive selection sites were found using FUBAR and MEME at 40 and 70, respectively. Using MEGA (Version 7.0) and operating the HyPhy software package, we found 18 sites under positive selection (Supplementary Table 1).

Discussion

This study was performed by the continuous monitoring of HRSV in children with SARI from 2018 to 2022, before and during the COVID-19 pandemic, in two sentinel hospitals in Zambia. To the best of our knowledge, this is the first study in Africa describing the evolutionary pattern of HRSV before and during the COVID-19 pandemic. The resurgence of HRSV cases after reduced transmission during the early phase of the pandemic has been reported in Australia and Austria [24,25]. A study in Italy and another in Australia analyzed the molecular characteristics of HRSV before and during the pandemic [24,26]. However, the evolutionary pattern behind the resurgence is unclear. We identified clades within HRSVA ON1 and HRSVB BA9 before and during the

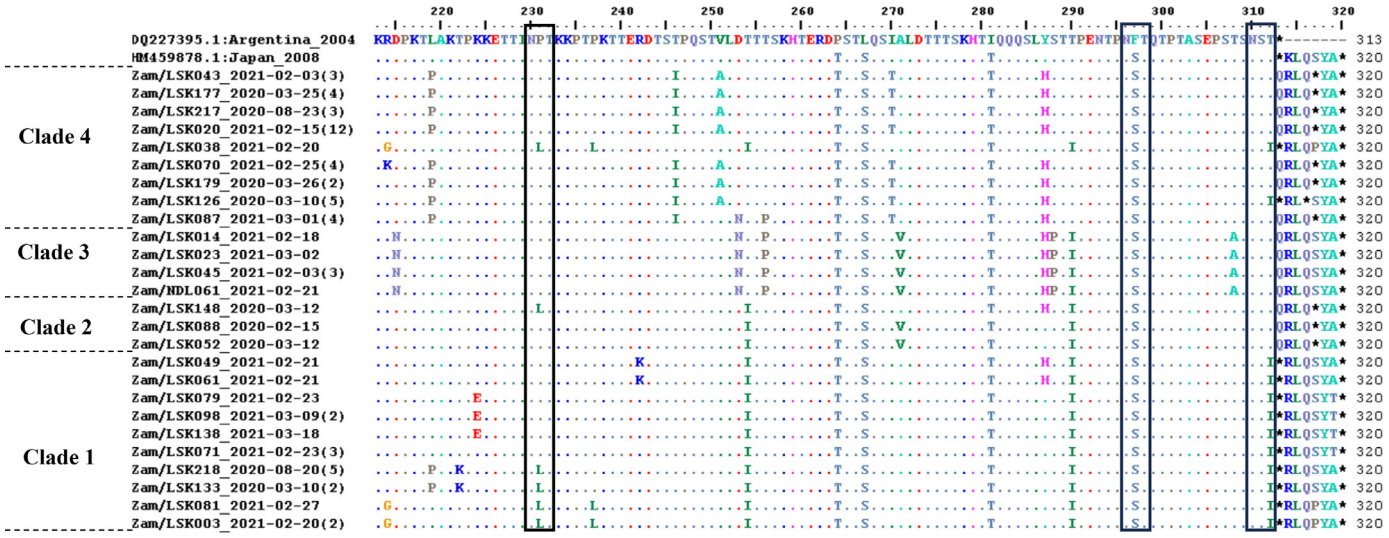

Figure 3b. HRSVB partial G gene amino acid substitutions deduced among SARI cases in 2018-2022 in Zambia. Strains from this study were compared with the reference DQ227395.1 (BA9) first described in Argentina. The number of sequences with additional identical strains is shown in parentheses on the right of the strain name. Dots represent identical residues. Asterisks represent stop codons. Potential N-linked glycosylation sites are indicated by horizontal black boxes. HRSV, human respiratory syncytial virus.

pandemic. We observed three patterns of different clades before and during the pandemic: new introductions, local persistence, and extinction. Clades 1 and 2 were unique to Zambia within HRSVA ON1 from 2018 to 2019. These clades were placed as separate clusters in the global data set, suggesting that they were introduced to the local population and persisted for some time. Notably, the strains in these clades did not appear after 2020, indicating the extinction of these unique clades. Two evolutionary patterns were obtained for Clade 5 during the pandemic: local persistence and new introductions. The Clade 5 strains in 2021 were almost identical to those identified in Ndola in 2019, indicating that this cluster locally persisted between 2019 and 2021. However, the cluster in 2022 was distinct from the locally persisted cluster, indicating that 2022 strains were newly introduced. For Clade 3, multiple introductions were detected from 2018 to 2022. The strains in 2022 did not cluster with strains detected in 2018 and 2019, suggesting that the 2022 strains were newly introduced. In the present study, all HRSVB strains were detected in 2020 and 2021, except for one strain detected in 2019. Therefore, it was not possible to analyze a long-term evolution pattern of HRSVB. Clade 1 of HRSVB had three distinct clusters: two clusters with 2021 stains and another cluster with 2020 strains, indicating the new introductions during the pandemic. Clade 4 of HRSVB had multiple clusters for 2020 and 2021 strains, but the largest cluster included strains from 2020 and 2021, indicating new introductions and persistence for Clade 4.

The resurgence of HRSV could be caused by new introductions or expansion of locally persistent strains. A study in Australia showed local expansion of persistent strains [24]. However, in Rome, Italy, new introductions and local persistence were responsible for the resurgence [26]. Although we observed the extinction of the unique Zambian clusters of HRSVA after the pandemic, some clades, such as Clade 5 of HRSVA and Clade 4 of HRSVB, showed a persistent pattern before and after the onset of the COVID-19 pandemic, indicating a low-level transmission of HRSV during the early phase of the pandemic. We observed similar levels of diversity before and after the resurgence of HRSV, as shown by the p-distance values in different years. This was because of many new introductions and local persistence during the pandemic. A similar pattern was seen in Rome, Italy [26]. Thus, there was no significant genetic bottleneck leading to lower genetic diversity.

A high level of HRSV admissions was observed in 2021 after the resurgence of HRSV. An increase in HRSV cases was also reported from other countries [24]. The resurgence in Zambia was caused by the cocirculation of multiple clades of HRSVA and HRSVB. In Zambia, HRSV is active usually from February to June. In 2020, HSRV activity increased in March when the first COVID-19 cases were identified in Zambia, but a few HRSV-positive cases were identified in April. Therefore, the outbreak in 2020 was smaller than the outbreaks in 2018 and 2019, most likely because of the control measures implemented during COVID-19. Early termination of the HRSV outbreak probably resulted in a low level of population immunity [13], leading to the cocirculation of HRSVA and HRSVB.

Glycosylation is necessary for evading host immunity and creating variations on antigenic sites [27]. During the COVID-19 pandemic, our study detected a loss in N-linked glycosylation at positions 320 in HRSVA and 312 in HRSVB. Changes in one or more glycosylation sites can significantly affect the virus's ability to survive and spread; small adjustments can affect the folding and conformation of the entire protein. Changes in N-glycosylation sites in the G gene were associated with increased HRSV severity [28]. Our study identified pervasive and episodic positive selection in the second hypervariable region of the G gene, which is a key determinant of HRSV evolution [29]. According to a study, amino acid substitutions in the G gene help the virus escape neutralizing antibodies [30].

Our study has several limitations. First, the study participants were enrolled from two major hospitals at two major cities in Zambia. The results may not provide a comprehensive picture of circulating HRSV strains in Zambia, particularly, in rural areas. Second, many HRSV-positive specimens were not typed and sequenced. This is because, after the initial reverse transcription-PCR screening, sequencing was only done after several months; this may have potentially impacted the sensitivity of specimens. In addition, specimens from ADH had to be shipped to the NIC in Lusaka for testing, which possibly impacted the specimen's integrity and quality owing to the lack of adequate cold chain facilities (Supplementary Table 2). In this regard, only ~40% of the 504 HRSV-positive samples were sequenced. This means that many positive samples could not sequence; therefore, these findings may not provide a comprehensive picture of genetic analysis and may exclude some potential strains of public health significance.

This study only targeted those specimens which were negative for either influenza and or SARS-CoV-2; However, this undertaking limits the chances of capturing more cases of HRSV, some studies have reported

that co-infection with HRSV and SARS-CoV-2 is uncommon [31,32]. Furthermore, only confirmed negative PCR results for influenza A or B and SARS-CoV-2 were considered for HRSV testing. This may potentially exclude positive HRSV cases in those with co-infection with influenza A or B and/or with SARS-CoV-2. Therefore, the number positive cases of HRSV reported in this study may be slightly high, although co-infection of HRSV/SARS-CoV-2, as reported in other studies, is rare and the dominance of influenza may delay the circulation of HRSV [33].

Despite the number of sequences recovered for either HRSVA or HRSVB being small, the overall outcome of the study on the extinction, introduction, and persistence of clades should not be affected because introductions or persistence and even extinctions may, in certain situations, just impact a small local population, as evidenced in Italy and Australia [24,26].

Despite these limitations, our study provides insights into the epidemiological pattern of HRSV before and during the COVID-19 pandemic in the African continent.

Conclusion

We identified a similar level of molecular diversity of HRSV with new introductions, local persistence, and extinction of certain clades before and during the pandemic. In 2021, a higher level of hospital admissions was observed than the pre-pandemic period because of the cocirculation of multiple clades of HRSVA and HRSVB. However, such unusual patterns were not seen in 2022, indicating the temporary effect of the pandemic on the transmission and molecular evolution of HRSV.

Declarations of competing interest

The authors have no competing interests to declare.

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Ethical approval

The Biomedical Research Ethics Committee, University of Zambia, School of Medicine (Version 691-2020), and the ethics committee of the Tohoku University Graduate School of Medicine, Sendai, Japan (Version 2019-1-639) approved the study protocol.

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Author contributions

Paul simusika, Michiko Okamoto and Hitoshi Oshitani developed the concept, analysed the data and wrote the manuscript. Evans Mwila, Clyde Dapat, Innocent Mwape, Moffat Malisheni,Mwaka Monze, Walter Muleya, Mayuko Saito, Takeaki Imamura and Sikandar Azam contributed to the writing of the Manuscript.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijregi.2024.03.009.

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