IJID Regions 8 (2023) 90–94

Contents lists available at ScienceDirect

IJID Regions

journal homepage: www.elsevier.com/locate/ijregi

Respiratory viruses in rural Zambia during the second year of the COVID-19 pandemic

Catherine G. Sutcliffe[a,b,*], Mutinta Hamahuwa[c], Evan Miller[d], Pamela Sinywimaanzi[c], Justin Hardick[d], Juliet Morales[b,#], Passwell Munachoonga[c], Mwaka Monze[e], Yukari C. Manabe[d,f], Katherine Z.J. Fenstermacher[g], Richard E. Rothman[g], Andrew Pekosz[d,f,g], Philip E. Thuma[c,f], Edgar Simulundu[c]

[a] Johns Hopkins University Bloomberg School of Public Health, Department of Epidemiology, Baltimore, USA
[b] Johns Hopkins University Bloomberg School of Public Health, Department of International Health, Baltimore, USA
[c] Macha Research Trust, Choma, Zambia
[d] Johns Hopkins University School of Medicine, Division of Infectious Diseases, Baltimore, USA
[e] University Teaching Hospital, Virology Laboratory, Lusaka, Zambia
[f] Johns Hopkins University Bloomberg School of Public Health, Department of Microbiology and Immunology, Baltimore, USA
[g] Johns Hopkins University School of Medicine, Department of Emergency Medicine, Baltimore, USA

A R T I C L E I N F O

Keywords:
Surveillance
Africa
Respiratory infections
SARS-CoV-2
Influenza
Respiratory syncytial virus

A B S T R A C T

Objectives: Limited data on respiratory infections are available from sub-Saharan Africa during the COVID-19 pandemic. The objective of this study was to evaluate the burden of respiratory viruses in rural Zambia from 2019-2021.

Methods: Surveillance was initiated at Macha Hospital in Zambia in December 2018. Each week, patients with respiratory symptoms were enrolled from the outpatient clinic. Nasopharyngeal samples were collected and tested for respiratory pathogens. The prevalence of respiratory symptoms and viruses in 2021 was compared to results from 2019 and 2020.

Results: After seeing few cases of influenza virus and respiratory syncytial virus in 2020, a return to prepandemic levels was observed in 2021. Rhinovirus/enterovirus, parainfluenza virus 1-4, and adenovirus circulated from 2019 to 2021, while human metapneumovirus and human coronaviruses (HKU1, 229E, OC43, and NL63 subtypes) were observed sporadically. SARS-CoV-2 was observed consistently in 2021 after being first identified in December 2020. The proportion of participants with co-infections in 2021 (11.6%) was significantly higher than in 2019 (6.9%) or 2020 (7.7%).

Conclusion: Declines in influenza virus and respiratory syncytial virus were reversed once public health measures were lifted. Respiratory viruses contributed to a significant burden of respiratory infections in 2021. This study provides important information about respiratory viruses in this changing context and underrepresented region.

Introduction

To control the spread of SARS-CoV-2, countries across the world implemented public health and social measures in 2020, including masking, quarantine and isolation, hand hygiene, and restrictions on travel and gatherings. These measures not only impacted transmission of SARS-CoV-2 but also other respiratory viruses [1], including influenza viruses and respiratory syncytial virus (RSV). In most settings, respiratory infections and viral detection decreased as transmission was interrupted in 2020 [1]. However, decreases were only temporary, with circulation resuming once measures were eased in late 2020. For seasonal viruses, such as RSV, off-season transmission or delayed seasons were observed in several settings in North America, Europe, and Asia [2–4].

Limited data are available from sub-Saharan Africa, despite children and adults in this region experiencing significant morbidity and mortality from respiratory infections [5]. Surveillance from South Africa and Zambia reported decreases in influenza viruses and RSV following implementation of public health measures in 2020 [6,7], but to date no studies have reported trends for respiratory infections following easing of restrictions.

* Corresponding author.
E-mail address: csutcli1@jhu.edu (C.G. Sutcliffe).
Present affiliation: HIV Surveillance Branch, Centers for Disease Control and Prevention

https://doi.org/10.1016/j.ijregi.2023.07.003
Received 18 April 2023; Received in revised form 5 July 2023; Accepted 6 July 2023

The Johns Hopkins Center for Excellence in Influenza Research and Surveillance (JHCEIRS) established surveillance for respiratory infections in rural Zambia in 2018 to better understand the epidemiology of respiratory pathogens in an underrepresented region in global surveillance networks. Surveillance is ongoing, thereby providing the opportunity to compare the respiratory pathogen landscape before and during the COVID-19 pandemic in this area. The objective of this analysis was to evaluate the burden and patterns of respiratory viruses in rural Zambia in 2021 compared to previously reported data from 2019 and 2020 [7,8].

Methods

Study setting, participants, and procedures

In December 2018, respiratory surveillance was established at Macha Hospital in Southern Province, Zambia. The hospital is located in a rural area populated primarily by subsistence farmers and provides care for a catchment population of over 150,000 individuals.

The surveillance methodology has been previously described [9]. Briefly, all patients attending the outpatient department were screened for influenza-like illness (ILI), which was defined as documented (≥38°C) or self-reported fever in combination with cough or sore throat, with onset or worsening in the past 7 days. On a weekly basis, an age-stratified (<1, 1-4, 5-15, 16-50, and ≥51 years) sample (target n = 16) of outpatients with ILI were approached for enrollment. At the enrollment visit, study staff administered a questionnaire to collect information on demographics, household composition, symptoms, and medical history. A nasopharyngeal swab (*Copan Diagnostics Inc*., Murrieta, California) was also collected and placed immediately in universal transport media (*Copan Diagnostics Inc.*, Murrieta, California).

Specimen testing and selection

After collection, nasopharyngeal swabs were taken to the Macha Research Trust Clinical Research Laboratory for testing using the GeneXpert Xpress Flu/RSV/SARS-CoV-2 assay (*Cepheid,* Sunnyvale, California). Samples were then shipped to Johns Hopkins University and tested with the BioFire® FilmArray Respiratory Panel EZ (*BioFire Diagnostics,* Salt Lake City, Utah) in 2019 and the ePlex Respiratory Pathogen Panel 2 (*GenMark Diagnostics, Inc*. Carlsbad, California) in 2020 and 2021. These panels detect adenovirus, human metapneumovirus, human rhinovirus/enterovirus, influenza A virus, influenza B virus, parainfluenza virus 1-4, RSV, coronaviruses (HKU1, 229E, OC43, and NL63 subtypes, but not SARS-CoV-2), SARS-CoV-2, *Chlamydia pneumoniae*, and *Mycoplasma pneumoniae*. Due to limited availability of ePlex cartridges, a random sample of swabs was selected from each month for testing in 2020 (n = 346 of 476) and 2021 (n = 251 of 417).

Statistical methods

Respiratory infections in Macha from December 2018 to December 2020 have been previously reported [7–9]. For this analysis, temporal trends in respiratory infections and the prevalence of individually detected viruses (no cases of *Chlamydia pneumoniae* or *Mycoplasma pneumoniae* were detected) were described among outpatients from January 1, 2021, to December 31, 2021. Results from 2019 and 2020 are provided for comparison.

Temporal trends in outpatient visits and ILI were summarized overall and by age group using descriptive statistics and graphical representations, including locally weighted scatterplot smoothing methods. Patient volume and the prevalence of ILI were compared across years using one-way analysis of variance and age-adjusted log-binomial regression, respectively.

Characteristics of the study population were summarized and compared between years using chi-square tests for categorical characteristics and Kruskal-Wallis test for continuous characteristics (Supplemental Table 1).

The prevalence of respiratory viruses in the outpatient population with ILI was estimated by month and year. Survey sampling weights were used in estimating the prevalence of each virus to account for the sampling strategy used to enroll participants and test specimens. For all viruses, weights were calculated based on the monthly age distributions of the outpatient population with ILI. As testing for adenovirus, parainfluenza virus 1-4, human metapneumovirus, human rhinovirus/enterovirus, and human coronaviruses (non-SARS-CoV-2) was

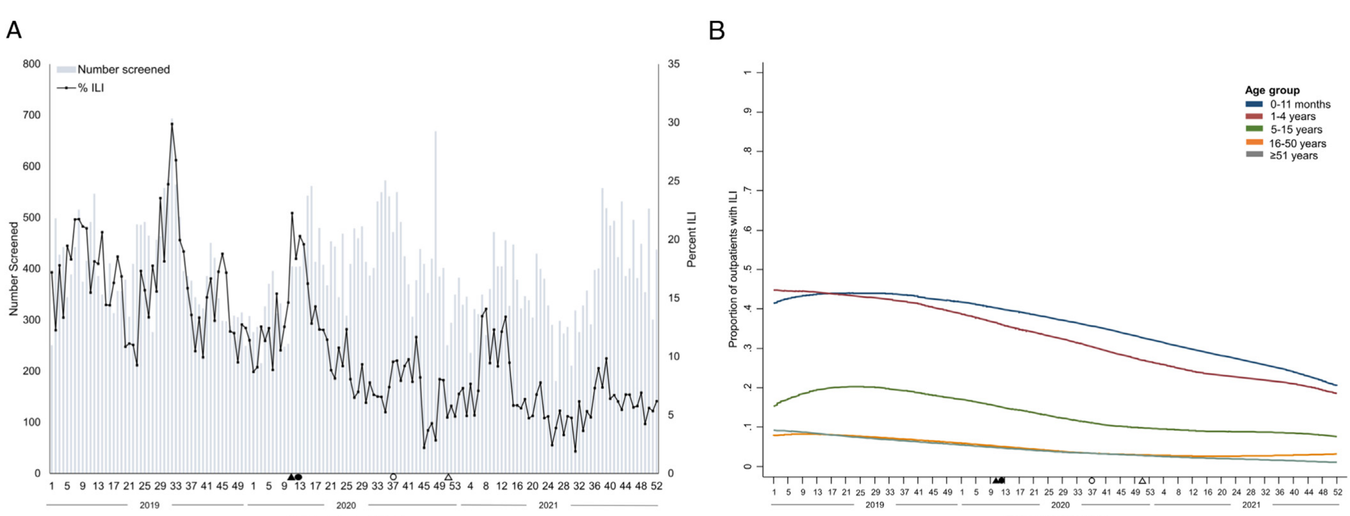

Figure 1. (a) Outpatient attendance and prevalence of ILI by week and (b) prevalence of ILI by week and age group among outpatients at Macha Hospital, 2019 to 2021. ILI: influenza-like illness. Solid triangle = World Health Organization declares COVID-19 a pandemic on March 11, 2020; Solid oval = first SARS-CoV-2 infection identified in Zambia on March 18, 2020 and implementation of mitigation measures on March 26, 2020; Hollow oval = easing of most restrictions related to travel, gatherings, restaurant/gym/bar closures, school closures on September 11, 2020 (restrictions were eased in a phased manner starting on April 24, 2020 with resumption of congregation at places of worship, non-contact sports, and operation of salons and barbershops; on May 8, 2020, restaurants, cinemas, gyms, and casinos reopened, and hotels, lodges, tour operators and other businesses that closed voluntarily advised to consider reopening; on June 1, 2020, primary and secondary schools reopened for examination classes only; on June 8, 2020, colleges and universities began a phased reopening for final year students only; on June 25, 2020, all international airports reopened); Hollow triangle = first SARS-CoV-2 infection identified in Macha on December 18, 2020.

only carried out on a subset of samples, additional weights were calculated based on the monthly distribution of samples selected for testing.

Co-infections were evaluated among samples tested with the BioFire/ePlex assay, and defined as the detection of two or more viruses. Age-adjusted log-binomial regression was used to compare the proportion with co-infections.

Statistical analyses were conducted using Stata Statistical Software, version 14 (StataCorp LLC., College Station, Texas) and SAS/STAT software, version 9.4 (SAS Institute Inc., Cary, North Carolina).

Research ethics and consent

The surveillance was approved by the Institutional Review Boards at Johns Hopkins (IRB00168163) and Macha Research Trust (E.2018.02), and the Zambian National Health Research Authority. Written informed consent for participation was obtained from adult participants and the parents or legal guardians of pediatric participants. Written assent was obtained from children 12 to 15 years of age.

Results

ILI surveillance

In 2021, there were 19,319 outpatient visits (monthly average: 1610 patients). This was a nonsignificant decrease in patient volume compared to 2019 (n = 21,453; monthly average: 1788 patients) and 2020 (n = 20,963; monthly average: 1747 patients), with lower volumes primarily observed in the middle of the year. The prevalence of ILI decreased significantly from 16.9% in 2019 to 6.9% in 2021 (Figure 1a), with decreases observed primarily among children (Figure 1b).

The majority of all outpatient visits were for adults 16-50 years of age (57.8% in 2021; Supplemental Figure 1A), while the majority of outpatient visits with ILI were for children 0-4 years (57.3% in 2021; Supplemental Figure 1B). The age distribution of outpatients with ILI did not significantly change from 2019 to 2021.

Respiratory virus prevalence

After seeing no cases of influenza A and B viruses and very few cases of RSV in 2020, these viruses returned in 2021 with similar patterns as in 2019 (Figure 2; Supplemental Figure 2). Compared to 2019, there was a significantly lower prevalence of influenza A virus, higher prevalence of influenza B virus, and similar prevalence of RSV in 2021. Rhinovirus/enterovirus, parainfluenza virus, and adenovirus circulated throughout 2019-2021. There was a significant increase in prevalence of rhinovirus/enterovirus in 2020 across all age groups (Supplemental Figure 2), particularly during the early months of the pandemic. There was a significant increase in prevalence of parainfluenza virus 1-4 in 2020 and 2021 compared to 2019, primarily among children 0-4 years of age.

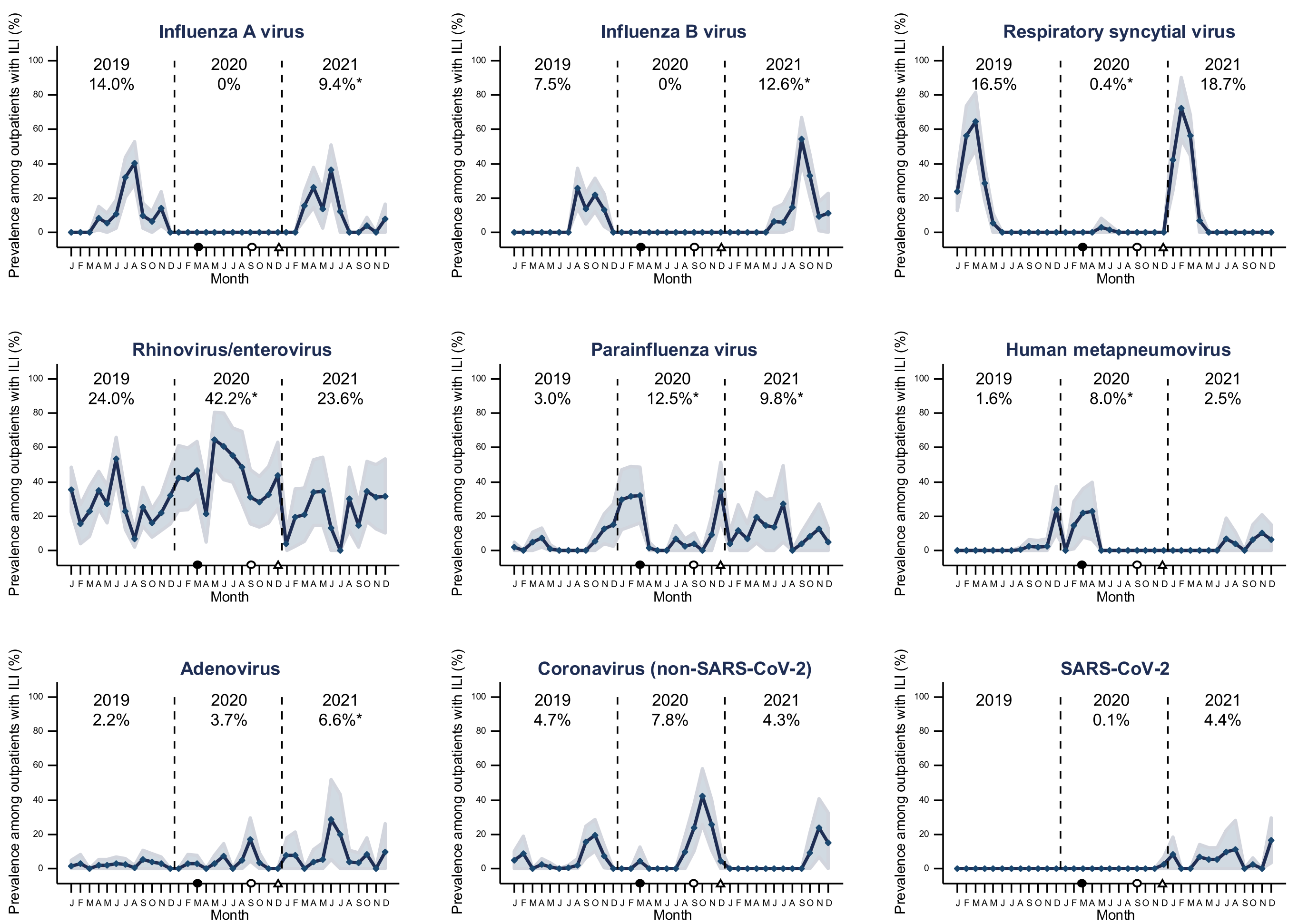

Figure 2. Prevalence of respiratory viruses among outpatients with ILI at Macha Hospital, 2019 to 2021. ILI: influenza-like illness. *$P<0.05$ for comparison between annual weighted prevalence in 2020 or 2021 and 2019 using a Rao-Scott chi-square goodness-of-fit test. Dashed lines = demarcation between calendar years; Blue solid line = monthly weighted prevalence of each pathogen; Gray shading around solid line = 95% confidence interval for the monthly weighted prevalence; Solid oval = World Health Organization declares COVID-19 a pandemic on March 11, 2020; First SARS-CoV-2 infection identified in Zambia on March 18, 2020, and implementation of mitigation measures on March 26, 2020; Hollow oval = easing of most restrictions related to travel, gatherings, restaurant/gym/bar closures, school closures on September 11, 2020; Hollow triangle = first SARS-CoV-2 infection identified in Macha on December 18, 2020.

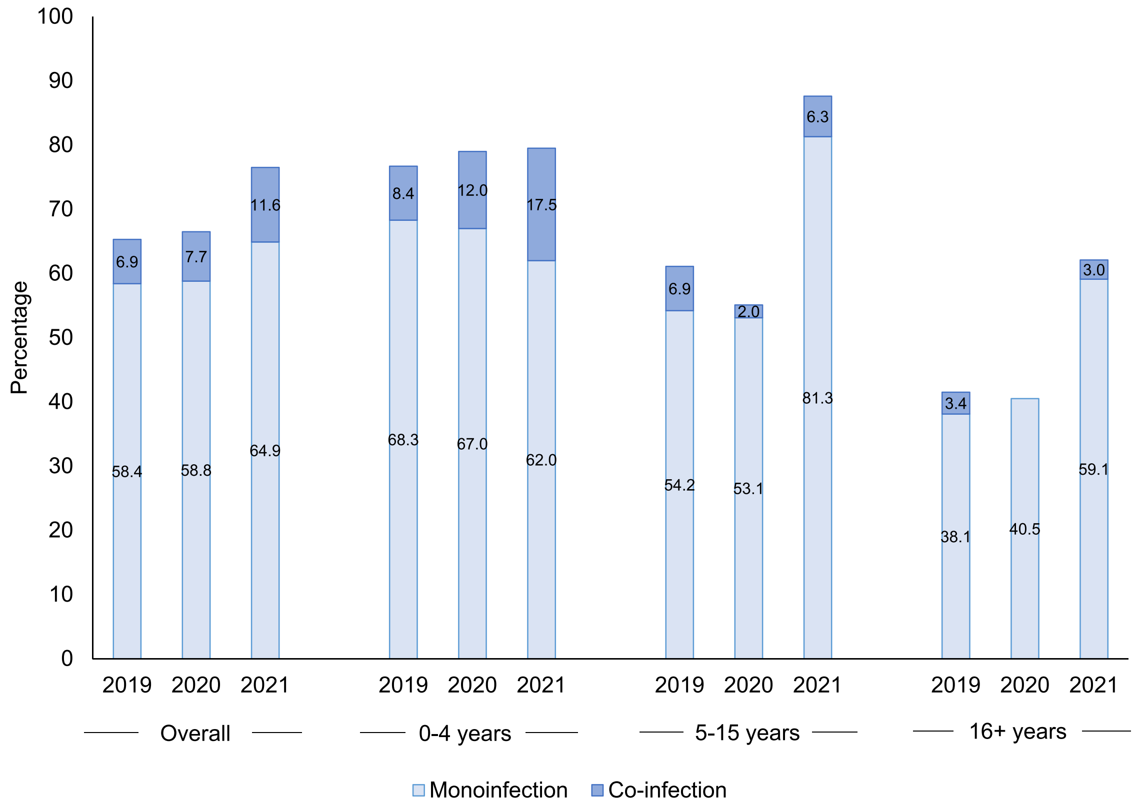

Figure 3. Mono and co-infections among outpatients with influenza-like illness at Macha Hospital, 2019 to 2021. Note: The analysis was restricted to samples tested using the BioFire (2019) or ePlex (2020 and 2021) respiratory panels. Co-infection was defined as the concurrent detection of two or more viruses.

A significant increase in prevalence of adenovirus was also observed in 2021, primarily among children 0-4 years of age. Human metapneumovirus and human coronaviruses (non-SARS-CoV-2) were observed sporadically throughout 2019-2021. A significant increase in prevalence of human metapneumovirus was seen in 2020 but not 2021, primarily among older children and adults. No significant differences in prevalence of human coronaviruses were seen across years, although the overall prevalence was higher in 2020 due to a peak at the end of the year among all age groups. SARS-CoV-2 was first identified in Macha in December 2020 and then consistently throughout 2021 with peaks in January, July/August, and December. The prevalence of SARS-CoV-2 was highest among adults (Supplemental Figure 2).

Viral co-infections

In 2021, 76.5% (n = 192/251) of participant samples had at least one virus detected and 11.6% (n = 29/251) had co-infections, which was significantly higher than in 2019 (6.9%; n = 38/553) or 2020 (7.7%; n = 25/323). Co-infections were most prevalent among children 0-4 years of age in all the years (Figure 3; see Supplemental Table 2 for participant characteristics). The most common co-infections in 2021 were rhinovirus/enterovirus plus parainfluenza virus (n=6) and rhinovirus/enterovirus plus adenovirus (n = 6), similar to 2020 (Supplemental Figure 3). In contrast, the most common co-infection was rhinovirus/enterovirus plus RSV (n = 12) in 2019.

Discussion

In this rural area of southern Zambia, respiratory viruses, including SARS-CoV-2, were circulating in 2021 and contributed to a significant burden of respiratory infections among outpatients.

The first case of COVID-19 in Macha was identified in December 2020, despite circulation of SARS-CoV-2 throughout Zambia in 2020. By November 2020, over 17,000 cases of COVID-19 were reported nationally and approximately 1900 cases were reported in Southern Province [10,11]. Public health measures were implemented within days of detecting the first case in March 2020, including restrictions on gatherings and domestic and international travel. Restrictions were lifted in phases starting in April 2020, with international airports reopening in June 2020 and schools reopening for regular classes in September 2020 (Figure 1) [7,12]. As Macha is a rural area connected to other towns by only a few roads, SARS-CoV-2 required introduction through travel which did not consistently happen until restrictions were eased. The first cases occurred in Macha among healthcare workers, several of whom had a history of recent domestic travel [13]. The virus then spread quickly into the community. In 2021, an increase in prevalence among outpatients mid-year coincided with a peak in cases nationally [11] and introduction of the Delta SARS-CoV-2 variant into the community [13]. During this time, visits to the outpatient department decreased, potentially due to concerns about traveling or attending healthcare facilities and public health activities (e.g., contact tracing) in the community [14]. The prevalence increased again at the end of 2021, coinciding with introduction of the Omicron SARS-CoV-2 variant.

Similarly, for seasonal viruses, such as influenza viruses and RSV, public health measures interrupted transmission, with no cases of influenza viruses and only a few cases of RSV seen in 2020, a phenomenon that has now been described in many settings [1]. With resumption of gatherings and travel, these viruses returned in 2021 with patterns of transmission similar to 2019 before the pandemic. Off-season transmission, which was observed in several settings [2–4], was not observed in this setting after most measures were lifted in Zambia in September 2020, perhaps due to resumption of travel and gatherings relatively close in time to when they were found to circulate before the pandemic. For rhinovirus/enterovirus, parainfluenza virus, and adenovirus, seasonal trends were not observed in this setting and these viruses were less impacted by implementation and easing of public health measures, with detections identified throughout 2020 and 2021 at higher levels than before the pandemic. This has been observed in other settings, particularly for rhinovirus/enterovirus [15], and may be due to several factors, including circulation of these viruses in the community at the onset of the pandemic, viral properties that render them more stable in the environment and more resistant to disinfectants, and fomite transmission rendering masking less effective [7,16].

This study had some limitations. First, surveillance was carried out at a health facility and therefore only captured medically-attended symptomatic disease which underestimates community-level burden of disease. In addition, the surveillance methods cannot account for changes in health-seeking behavior, which may be important during a pandemic if individuals avoid health facilities. There was a decrease in overall visits in mid-2021 during the Delta wave as well as a shift toward older age groups among all outpatients in 2020 and 2021, suggesting some impact on health-seeking behavior. These changes may also have led to the burden of disease being underestimated. Second, only a subset of samples were tested for the full panel of viruses. While a random sampling strategy was used to select samples for testing, the relatively small number of specimens from adult age groups may have resulted in missed infections, thus underestimating the true burden of disease among adults. Finally, surveillance was conducted in a single health facility in a rural area of Zambia which may not be representative of all rural areas in the country or region.

In summary, the dramatic decline in influenza viruses and RSV observed in 2020 in this rural area was reversed once public health measures were lifted, with patterns similar to those observed in 2019 before the pandemic. Other respiratory viruses continued to circulate in 2021 and contributed to a significant burden of respiratory infections. This ongoing surveillance platform provides important systematic information about respiratory viruses in this continuously changing context and in an underrepresented region of the world.

Declarations of competing interest

The authors have no competing interests to declare.

CRediT authorship contribution statement

Catherine G. Sutcliffe: Conceptualization, Methodology, Formal analysis, Supervision, Writing – original draft. **Mutinta Hamahuwa:** Project administration, Investigation, Data curation, Writing – review & editing. **Evan Miller:** Investigation, Writing – review & editing. **Pamela Sinywimaanzi:** Project administration, Data curation, Writing – review & editing. **Justin Hardick:** Investigation, Writing – review & editing. **Juliet Morales:** Project administration, Data curation, Writing – review & editing. **Passwell Munachoonga:** Investigation, Data curation, Writing – review & editing. **Mwaka Monze:** Supervision, Writing – review & editing. **Yukari C. Manabe:** Supervision, Writing – review & editing. **Katherine Z.J. Fenstermacher:** Supervision, Writing – review & editing. **Richard E. Rothman:** Supervision, Funding acquisition, Writing – review & editing. **Andrew Pekosz:** Supervision, Funding acquisition, Writing – review & editing. **Philip E. Thuma:** Supervision, Writing – review & editing. **Edgar Simulundu:** Supervision, Writing – review & editing.

Funding

This work was supported by the National Institute of Allergy and Infectious Diseases (contract HHSN272201400007C) awarded to the Johns Hopkins Center of Excellence in Influenza Research and Surveillance (JHCEIRS), (contract HHSN7593021C00045) awarded to the Johns Hopkins Center of Excellence in Influenza Research and Response (JHCEIRR), and the Richard Eliasberg Family Foundation. Any opinions, findings, conclusions, or recommendations expressed are those of the authors and do not represent the policy or position of National Institute of Allergy and Infectious Diseases or National Institutes of Health. The funding agency had no role in the analysis and interpretation of the data, the writing of the manuscript, or the decision to submit the manuscript for publication.

Ethical approval statement

The surveillance was approved by the Institutional Review Boards at Johns Hopkins (IRB00168163) and Macha Research Trust (E.2018.02), and the Zambian National Health Research Authority. Written informed consent for participation was obtained from adult participants and the parents or legal guardians of pediatric participants. Written assent was obtained from children 12 to 15 years of age.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijregi.2023.07.003.

References

[1] Achangwa C, Park H, Ryu S, Lee MS. Collateral impact of public health and social measures on respiratory virus activity during the COVID-19 pandemic 2020–2021. *Viruses* 2022;**14**. doi:10.3390/v14051071.

[2] Perez A, Lively JY, Curns A, Weinberg GA, Halasa NB, Staat MA, et al. Respiratory virus surveillance among children with acute respiratory illnesses – New Vaccine Surveillance Network, United States, 2016–2021. *MMWR Morb Mortal Wkly Rep* 2022;**71**:1253–9. doi:10.15585/mmwr.mm7140a1.

[3] Ujiie M, Tsuzuki S, Nakamoto T, Iwamoto N. Resurgence of respiratory syncytial virus infections during COVID-19 pandemic, Tokyo, Japan. *Emerg Infect Dis* 2021;**27**:2969–70. doi:10.3201/eid2711.211565.

[4] Fourgeaud J, Toubiana J, Chappuy H, Delacourt C, Moulin F, Parize P, et al. Impact of public health measures on the post-COVID-19 respiratory syncytial virus epidemics in France. *Eur J Clin Microbiol Infect Dis* 2021;**40**:2389–95. doi:10.1007/s10096-021-04323-1.

[5] Iuliano AD, Roguski KM, Chang HH, Muscatello DJ, Palekar R, Tempia S, et al. Estimates of global seasonal influenza-associated respiratory mortality: a modelling study. *Lancet* 2018;**391**:1285–300. doi:10.1016/S0140-6736(17)33293-2.

[6] Olsen SJ, Azziz-Baumgartner E, Budd AP, Brammer L, Sullivan S, Pineda RF, et al. Decreased influenza activity during the COVID-19 pandemic - United States, Australia, Chile, and South Africa, 2020. *MMWR Morb Mortal Wkly Rep* 2020;**69**:1305–9. doi:10.15585/mmwr.mm6937a6.

[7] Loevinsohn G, Hamahuwa M, Hardick J, Sinywimaanzi P, Fenstermacher KZJ, Munachoonga P, et al. Respiratory viruses in rural Zambia before and during the COVID-19 pandemic. *Trop Med Int Health* 2022;**27**:647–54. doi:10.1111/tmi.13781.

[8] Loevinsohn G, Hardick J, Sinywimaanzi P, Fenstermacher KZJ, Shaw-Saliba K, Monze M, et al. Respiratory pathogen diversity and co-infections in rural Zambia. *Int J Infect Dis* 2021;**102**:291–8. doi:10.1016/j.ijid.2020.10.054.

[9] Loevinsohn G, Hamahuwa M, Sinywimaanzi P, Fenstermacher KZJ, Shaw-Saliba K, Pekosz A, et al. Facility-based surveillance for influenza and respiratory syncytial virus in rural Zambia. *BMC Infect Dis* 2021;**21**:986. doi:10.1186/s12879-021-06677-5.

[10] ZNPHI and Ministry of Health. Zambia COVID-19 dashboard, https://nsdi-mlnr.maps.arcgis.com/apps/dashboards/c08c4cce115244f7ba472a458e1483f7; 2021 [accessed 15 June 2021].

[11] World Health Organization. Coronavirus (COVID-19) Dashboard, https://covid19.who.int/; 2021 [accessed 15 May 2021].

[12] ZNPHI. Situation reports, (COVID-19) SITREPS. Coronavirus Disease, http://znphi.co.zm/news/situation-reports-new-coronavirus-covid-19-sitreps/; 2019 [accessed 15 June 2021].

[13] Sinywimaanzi P, Sianyinda M, Hamahuwa M, Munachoonga P, Muleka M, Morales J, et al. Clinical outcomes among individuals infected with SARS-CoV-2 in rural Zambia: a longitudinal study *American Society for Tropical Medicine and Hygiene Annual Meeting Seattle, Washington*; October 30-November 3, 2022. Abstract # 1136.

[14] Sutcliffe CG, Sinywimaanzi P, Morales J, Sianyanda M, Muleka M, Fenstermacher KZJ, et al. Uptake of public health measures and vaccine acceptance during the COVID-19 pandemic in rural Zambia. *Hum Vaccin Immunother* 2022;**18**:2153538. doi:10.1080/21645515.2022.2153538.

[15] Teo KW, Patel D, Sisodia S, Roland D, Gaillard EA, Tang JW. Rhinovirus persistence during the COVID-19 pandemic-Impact on pediatric acute wheezing presentations. *J Med Virol* 2022;**94**:5547–52. doi:10.1002/jmv.27986.

[16] Oh DY, Buda S, Biere B, Reiche J, Schlosser F, Duwe S, et al. Trends in respiratory virus circulation following COVID-19-targeted nonpharmaceutical interventions in Germany, January–September 2020: analysis of national surveillance data. *Lancet Reg Health Eur* 2021;**6**:100112. doi:10.1016/j.lanepe.2021.100112.