>50 years (24.3%), p<0.0001. In contrast, rhinoviruses were most prevalent in children <5 years (28.7%), followed by children aged 5-17 (15.8%), adults aged 18-50 (8.3%) and >50 years (6.3%), p<0.0001. Four SARS-CoV-2 waves occurred, with 36.1%-52.4% SARS-CoV-2 positivity during peak months. Influenza infections were observed in both 2020 and 2021 during the rainy season as expected (peak positivity 16.4%-23.5%). Peaks of rhinovirus were asynchronous to the months when SARS-CoV-2 and influenza peaked.

Conclusion: Our data show that many respiratory viruses continued to circulate during the COVID-19 pandemic in The Gambia, including human rhinoviruses, despite the presence of NPIs during the early stages of the pandemic, and influenza peaks during expected months.

Keywords
SARS-CoV-2, respiratory viruses, surveillance, Influenza like illness

This article is included in the Coronavirus (COVID-19) collection.

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How to cite this article: Jarju S, Senghore E, Brotherton H *et al.* **Circulation of respiratory viruses during the COVID-19 pandemic in The Gambia [version 1; peer review: 2 approved]** Gates Open Research 2022, **6**:148 https://doi.org/10.12688/gatesopenres.14155.1

First published: 08 Dec 2022, **6**:148 https://doi.org/10.12688/gatesopenres.14155.1

Introduction

Many countries reported a dramatic reduction in the circulation of common respiratory viruses such as influenza and respiratory syncytial virus (RSV) during the initial stages of the COVID-19 pandemic[1–3]. This was attributed to the multiple non-pharmaceutical interventions (NPIs) implemented to reduce SARS-CoV-2 transmission such as bans on travel and social interactions, school closures, and mandatory wearing of face masks. Many of these early studies are from high-income countries with temperate climates, where the transmission patterns and seasonality of influenza and other respiratory viruses are well established[1–3]. In West Africa, surveillance for respiratory viruses is limited and data on the circulation of influenza and other viruses during the COVID-19 pandemic scarce.

The Gambia reported its first COVID-19 case on the 17th March 2020 in a traveller[4]. A state of public emergency was declared on 27th March 2020: NPIs such as social distancing, borders closure, closure of schools, ban on public gatherings, a night-time curfew, and compulsory wearing of face masks in public places were implemented. No increase in SARS-CoV-2 cases were seen in The Gambia until June 2020, when the first wave of infections occurred[5]. The state of emergency was relaxed on the 17th September 2020 and since then, NPIs have not been enforced. Nevertheless, three further SARS-CoV-2 waves have occurred in The Gambia up to March 2022[5].

We conducted a surveillance of respiratory viruses, including SARS-CoV-2, between April 2020 and March 2022. Our objectives were to describe the extent to which other respiratory viruses circulated alongside SARS-CoV-2 in The Gambia during the study period, whether there were differences in the prevalence of SARS-CoV-2 and other respiratory viruses across age groups, and whether peaks of other viruses such as human rhinovirus occurred at the same time or asynchronously with SARS-CoV-2.

Methods

Study setting, participant recruitment and sample collection

The Gambia has a population of approximately 2.5 million, with more than half living in urban coastal areas[6]. The climate is subtropical with a short rainy season between June and October, and a long dry season for the rest of the year[7]. Average temperatures range between 23°C to 33°C during the rainy season, and between 18°C to 30°C during the dry season[7]. Knowledge of influenza virus circulation is limited but coincided with the rainy season in a 12-month surveillance study[8]. No routine influenza vaccination programme is in place in The Gambia. SARS-CoV-2 vaccination of adults commenced in March 2021.

Nasopharyngeal swabs (NPS) from two surveillance studies were used for this analysis. In both studies, we received written consent from participants. Where the participants were children, consent was sought from parents/guardians and assent of the subject. In the first study, influenza-like illness (ILI) surveillance samples were collected from adults and children of all ages with acute respiratory illnesses attending the outpatient medical services at the Medical Research Council Unit The Gambia at the London School of Hygiene and Tropical Medicine (MRCG@LSHTM) clinics in Fajara (urban, coastal) and Keneba (rural) as shown in Figure 1. Patients presenting with shortness of breath, cough, or history of fever of any severity within the last 10 days provided written informed consent prior to study recruitment. Sample collection was carried out between April 2020 and June 2021 in Fajara and between September 2020 and June 2021 in Keneba.

In the second study, samples were collected between January 2021 and March 2022 from a clinical trial on COVID-19 treatment conducted in urban and peri-urban areas from sites shown in Figure 1 (Brikama, Bundung, Bakau and Fajara), the PaTS study; Prevention and Treatment for Covid-19 associated severe pneumonia in The Gambia: a Randomized Clinical Trial (NCT04703608). At screening, written informed consent was obtained from potential participants aged ≥5 years presenting with acute fever and cough, or three or more of the following signs or symptoms: fever, cough, general weakness/fatigue, headache, myalgia, sore throat, coryza, dyspnoea, anorexia/nausea/vomiting, diarrhoea, recent loss of smell, recent loss of taste, altered mental status. Data on self-reported sex, or in the case of children, parent-reported sex, was also collected.

Both studies were approved by the Gambia Government and MRCG joint ethics committee and the Research Ethics

Figure 1. A map of The Gambia showing sites where participants were recruited for the two studies. The PaTS study sites were in Brikama, Bundung, Bakau and Fajara. The influenza-like illness (ILI) study sites were in Keneba and Fajara.

Committee at the London School of Hygiene and Tropical Medicine (LSHTM).

Respiratory virus detection

Ribonucleic acid (RNA) was extracted from 200μL of universal transport medium from NPS using the QIAamp Viral RNA kit (QIAGEN, Germany) as per the manufacturer's instructions. All samples were spiked with Phocine herpes virus (PhHV) prior to extraction as a quality control for RNA extraction efficiency.

SARS-CoV-2 was detected using a Reverse Transcriptase Polymerase Chain Reaction (RT-PCR), with either the Takara One Step PrimeScript III RT-PCR Kit (TaKaRa Bio Inc., Japan, catalogue number RR600A) or the SuperScript III Platinum One-Step qRT-PCR Kit (Invitrogen, USA, catalogue number 11732-088). Primers and probes targeting SARS-CoV-2 E and N genes, along with RNase P and PhHV were used previously described[4]. Samples were considered positive if all four gene targets amplified at a cycle threshold (Ct) of 37 or below. In addition, a viral multiplex RT-PCR assay was used as previously described[8] to detect influenza A and B, respiratory syncytial virus (RSV) A and B, parainfluenza viruses 1–4, human metapneumovirus (HMPV), adenovirus, seasonal coronaviruses (229E, OC43, NL63) and human rhinovirus. All samples with a Ct of ≤37 and passed quality control measures were considered positive.

Data analysis

Clinical data, demographics and RT-PCR results were entered on a REDCap database. All results and metadata were downloaded from the database and analysed using Graphpad Prism V9.1.2 or Microsoft Excel. Statistical comparisons between group proportions were carried out using the two-sided chi-squared or Fisher's exact test.

Results

Among the 1397 participants recruited between April 2020 and March 2022, 617 (44.2%) were positive for one or more respiratory viruses. Virus positivity was significantly higher in children <5 years (80.0%, 95% CI 71.9-86.9%) than in the other age groups: children aged 5–17 years (53.1%, 45.1-61.1%), adults aged 18–50 years (39.5%, 36.2-42.9%), and adults >50 years (39.9%, 34.0-46.1%), p<0.0001 (Table 1).

Overall, 255 (18.3%, 16.3-20.4%) of samples tested during the study period were positive for SARS-CoV-2 (Table 2). The next most prevalent viruses were human rhinovirus (10.5%, 8.9-12.2%) and influenza A or B (5.5%, 4.4-6.8%). SARS-CoV-2 positivity was lower among children <5 years (4.3%, 1.4-9.9%) than in children aged 5–17 years (12.7%, 7.9-18.9%), adults aged 18–50 (19.3%, 16.7-22.1%), and adults >50 years (24.3%, 19.3-29.8%), p<0.0001. In contrast, human rhinovirus infections were most prevalent in children aged <5 years (28.7%, 20.7-37.9%), than in children aged 5–17 years (15.8%, 10.5-22.3%), adults aged 18–50 (8.3%, 6.5-10.4%), and adults >50 years (6.3%, 3.7-10.0%), p<0.0001. Most RSV A and HMPV infections were detected in children <5 years old, although the number of cases was relatively low (Table 2). The prevalence of respiratory viruses within each age group across the different study sites is shown in Tables S1-S3.

Table 1. Recruited participants and percentage with respiratory viruses detected stratified by recruitment site, age and sex.
Total numbers are shown with % in brackets. The denominators for age and sex percentages are the total recruited numbers at each site or all participants combined. Virus positivity denotes detection of one or more viruses in the sample. The denominators for respiratory virus positive % are the total recruited within each sex or age strata for each study or all participants combined. The PaTS trial did not recruit any children below the age of 5 years.

		Fajara (n=611) (Apr 2020–Jun 2021)		Keneba (n=337) (Sep 2020–Jun 2021)		PaTS (n=449) (Jan 2021-Mar 2022)		All sites (n=1397) Apr 2020 – Mar 2022	
		Recruited participants	Virus positivity	Recruited participants	Virus positivity	Recruited participants	Virus positivity	Recruited participants	Virus positivity
Sex	Male	308 (50.4)	109 (35.4)	139 (41.2)	92 (66.2)	200 (44.5)	88 (44.0)	**647 (46.3)**	**289 (44.7)**
	Female	303 (49.6)	108 (35.6)	198 (58.8)	112 (56.6)	249 (55.5)	108 (43.4)	**750 (53.7)**	**328 (43.7)**
Age	< 5 years	50 (8.2)	30 (60.0)	65 (19.3)	57 (87.7)	0 (0.0)	0 (0.0)	**115 (8.3)**	**92 (80.0)**
	5–17 years	43 (7.0)	16 (37.2)	51 (15.1)	36 (70.6)	64 (14.3)	32 (50.0)	**158 (11.3)**	**84 (53.1)**
	18–50 years	401 (65.6)	134 (33.4)	145 (43.0)	77 (53.1)	310 (69.0)	127 (41.0)	**856 (61.2)**	**338 (39.5)**
	>50 years	117 (19.2)	37 (31.6)	76 (22.6)	34 (44.7)	75 (16.7)	36 (48.0)	**268 (19.2)**	**107 (39.9)**

Table 2. Respiratory viruses detected during the study period stratified by age of participants.
Respiratory viruses detected during the study period stratified by age of participants. The denominator
for virus positivity is total participants in the study (n=1397) or the number of participants recruited in
each age group. RSV A = Respiratory Syncytial Virus A; HMPV = Human Metapneumovirus; HPIV = Human
Parainfluenza viruses (pooled samples positive for HPIV1-4); Seasonal Coronavirus = combined 229E,
NL63 and OC43 positive samples. Influenza A = samples positive for influenza A but negative in the
pandemic H1N1 specific assay, which are presumed Influenza A H3N2, but not confirmed.

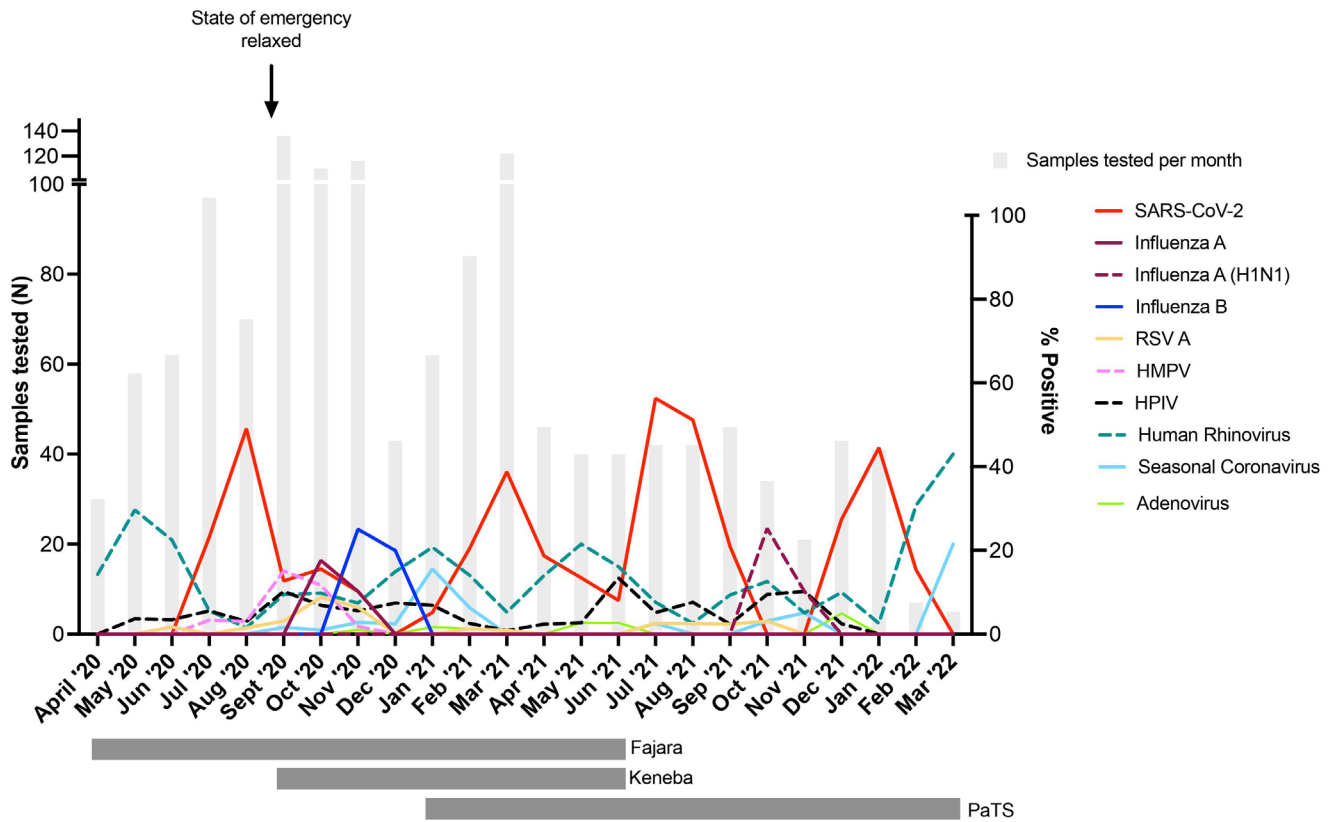|	Virus positivity (%)	<5 years	5–17 years	18–50 years	>50 years
SARS-CoV-2	255 (18.3)	5 (4.3)	20 (12.7)	165 (19.3)	65 (24.3)
Human Rhinovirus	146 (10.5)	33 (28.7)	25 (15.8)	71 (8.3)	17 (6.3)
Influenza (all)	77 (5.5)	10 (8.7)	15 (9.5)	42 (4.9)	10 (3.7)
Influenza A	29 (2.1)	4 (3.5)	7 (4.4)	12 (1.4)	6 (2.2)
Influenza A (H1N1)	10 (0.7)	0 (0.0)	2 (1.3)	8 (0.9)	0 (0.0)
Influenza B	38 (2.7)	6 (5.2)	6 (3.8)	22 (2.6)	4 (1.5)
HPIV	47 (3.4)	9 (7.8)	8 (5.1)	22 (2.6)	8 (3.0)
HMPV	38 (2.7)	14 (12.2)	9 (5.7)	13 (1.5)	2 (0.8)
RSV A	25 (1.8)	14(12.2)	2 (1.3)	9 (1.1)	0 (0.0)
Seasonal Coronavirus	26 (1.9)	2 (1.7)	4 (2.5)	15 (1.8)	5 (1.9)
Adenovirus	2 (0.1)	0 (0.0)	1 (0.6)	1 (0.1)	0 (0.0)
Samples tested	1397	115	158	856	268

Only 2.9% (41/1397) of recruited participants were hospitalized for their illness, although details of the clinical reasons for admission were not available. 15 (36.6%) of these admissions had respiratory viruses detected in NPS (3 RSV A, 3 Influenza B, 2 HMPV, 3 parainfluenza virus 1 and 4 human rhinovirus). 36.6% (15/41) of hospitalizations were in children <5 years old, representing a 13.0% (15/115) admission rate in this age group compared to 2.0% in the other age groups (26/1282), p<0.0001.

Four SARS-CoV-2 waves were observed in The Gambia during the 24-month study period (Figure 2); two during the rainy season (peak detection August 2020 and July 2021) and two during the dry season (peak detection March 2021 and January 2022). SARS-CoV-2 positivity during the four waves were 45.7%, 36.1%, 52.4% and 41.4%, respectively. Influenza A infections peaked in October 2020 (16.4% positivity) and October 2021 (23.5% positivity), with influenza B infections detected only in 2020 (peak November 2020, 23.3% positivity, Figure 2). Despite asynchronous peaks of SARS-CoV-2 and influenza, co-infections were observed, with 4/39 (10.3%) influenza A and 8/38 (21.1%) influenza B infected individuals also tested positive for SARS-CoV-2. 12.0% (3/25) of RSV A infections, 10.5% (4/38) of HMPV infections, 6.4% (3/47) of parainfluenza virus infections, and 4.1% (6/146) of human rhinovirus infections were also positive for SARS-CoV-2. Human rhinovirus positivity usually peaked at different times than SARS-CoV-2 and influenza (Figure 2). The prevalence of respiratory viruses over time stratified by study site is shown in Figure S1.

Discussion

SARS-CoV-2 was the most prevalent respiratory virus found among patients presenting with ILI during the COVID-19 pandemic in The Gambia. Although the seasonality of respiratory viruses is less predictable in tropical and sub-tropical than in temperate regions, many viral infections including influenza peak during the rainy season in The Gambia and neighbouring Senegal[8,9]. It is still unclear whether SARS-CoV-2 transmission will become seasonal, but The Gambia has experienced waves of infection in both the rainy and the dry season so far (twice per year), likely reflecting the emergence of new variants globally. Of note, the SARS-CoV-2 dynamics we have observed occurred in the context of no NPIs after the first wave and only 13.1% of the Gambian population fully vaccinated by February 2022[10].

We observed peaks in influenza activity in October during 2020 and 2021, although without robust surveillance data from previous years, it is difficult to determine whether there was a reduction in transmission. Our previous study in children <5 years old with ILI attending outpatient services in Fajara found 8.4% influenza A or B positivity over 12 months in 2018–19[8], compared to 7.3% in our current study which included both adults and children. In Senegal, between 1996 and 2009, 10.4% samples were positive for influenza A and 2.9% for influenza B[9]. Even in the absence of formal NPIs, either behavioural changes or reduction in global influenza transmission leading to reduced introductions could have an impact on influenza infections in The Gambia. However, the dramatic reductions in influenza seen in many high-income countries with strictly

Figure 2. Percentage of influenza-like illness cases positive for respiratory viruses in The Gambia during April 2020 – March 2022. Vertical grey bars indicate the number of samples tested per month. Date when COVID-19 pandemic state of emergency was lifted is shown, after which time no restrictions or non-pharmaceutical interventions were in place within the country. Horizontal grey bars under the graph show periods when sample collection was done in the three participating sites (Fajara, Keneba, PaTS Study). Surveillance in Fajara was carried out between April 2020 and June 2021. Surveillance in Keneba was carried out between September 2020 and June 2021. Sample collection in the PaTS study was between January 2021 and March 2022. RSV A = Respiratory Syncytial Virus A; HMPV = Human Metapneumovirus; HPIV = Human Parainfluenza viruses (pooled samples positive for HPIV1-4); Seasonal Coronavirus = combined 229E, NL63 and OC43 positive samples. Influenza A = samples positive for influenza A but negative in the pandemic H1N1 specific assay.

enforced NPIs do not appear to have occurred in The Gambia[1–3]. This may in part be due to poor adherence to NPIs, even when these measures were in place. In Canada, the average weekly positivity rate for influenza A in 2020–2021 was 0.012% compared to 10.40% in pre-pandemic years, and 0.006% for influenza B compared to 2.60% in pre-pandemic years[1]. Data from other African countries are scarce, with influenza and RSV reductions observed in South Africa[11], but not in the Democratic Republic of the Congo (5.6% influenza A positivity and 0.9% influenza B positivity Dec 2020 – March 2021)[12].

After SARS-CoV-2, human rhinovirus was the most prevalent virus detected in our samples. As expected, SARS-CoV-2 was responsible for a lower proportion of symptomatic ILI syndromes in children compared to adults, and the reverse pattern was seen for rhinovirus infections with decreasing prevalence with increasing age. Persistent circulation of human rhinoviruses has been described during the COVID-19 pandemic even in countries with NPI-driven reductions in other respiratory viruses, especially in the context of the

reopening of schools[1,13]. This was also evident in our data with rhinoviruses circulating during the only period of NPI enforced in The Gambia, although schools were closed during that period. The high prevalence of human rhinovirus infections in children and the resulting increases in upper respiratory tract infections have been proposed as a potential mechanism for the reduced severity of COVID-19 observed in children[14,15]. The shared mucosal niche provides the opportunity for viral-viral interactions and interference between influenza and human rhinoviruses has been demonstrated in both epidemiological and *in vitro* data[16,17]. Whether similar interference between rhinoviruses and SARS-CoV-2 occurs to a degree population level transmission dynamics of either virus are affected is currently unclear. Our data on rhinovirus peaks and troughs during the study period should be treated with caution due to the small sample size and lack of pre-pandemic annual rhinovirus circulation patterns. Nevertheless, our findings do suggest that asynchronous circulation of SARS-CoV-2 and rhinoviruses may occur, supporting the experimental data from recent studies[14,15].

This study has few limitations that need to be considered when interpreting our findings. The data come from two different studies with slightly different entry criteria, as well as different periods of participant recruitment. The main reasons for combining data from the two studies were to increase the sample size to enhance power and observe respiratory virus prevalence across a longer time period with which to arrive at more robust conclusions about seasonality. The PaTS trial did not recruit children under 5 years old, however, we show age stratified data to demonstrate any differences in respiratory virus prevalence across age groups. The PaTS trial also had a broader list of symptoms in the inclusion criteria. However, 454/484 (93.8%) participants included in the PaTS study had a history of fever or cough, therefore would have been recruited according to the ILI study inclusion criteria.

In summary, our data show that many respiratory viruses continued to circulate during the COVID-19 pandemic in The Gambia, including human rhinoviruses, despite the presence of NPIs during the early stages of the pandemic, and influenza peaks during expected months. The age distributions of SARS-CoV-2 and human rhinovirus prevalence in symptomatic ILI are diametrically opposite, with rhinovirus infections driving the greater respiratory virus positivity seen in young children. The degree to which virus-virus interactions shape the impact of future SARS-CoV-2 waves and seasonality is important to establish as the transition to endemicity occurs in the coming years.

Consent
Written informed consent for publication of the participants/patients' details was obtained from the participants/patients/parents/guardian/relative of the participant/patient.

Data availability
Underlying data
Open Science Framework: Circulation of respiratory viruses during the COVID-19 pandemic in the Gambia, https://doi.org/10.17605/OSF.IO/TSF78[18].

This project contains the following underlying data:

- EXTENDED DATA 2.xlsx (RT-PCR results for respiratory viruses detected from Fajara, Keneba and PaTS)

Extended data
Open Science Framework: Circulation of respiratory viruses during the COVID-19 pandemic in the Gambia, https://doi.org/10.17605/OSF.IO/TSF78[18].

This project contains the following extended data:

- EXTENDED DATA 1_TdS.docx (Table S1. Respiratory viruses detected during the study period stratified by age of participants for Fajara site)

Data are available under the terms of the Creative Commons Zero "No rights reserved" data waiver (CC0 1.0 Public domain dedication).

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http://www.doi.org/10.17605/OSF.IO/TSF78

Open Peer Review

Current Peer Review Status: ✓ ✓

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x

The manuscript is well written, and contains important data. It has a number of limitations, i.e. limited demographic and clinical data, differing recruitment periods and eligibility criteria. The latter points are acknowledged by the authors.

Comments:
1. Local SARS-CoV-2 and influenza vaccination policy should be stated in the Methods.

2. More detail on the PaTS trial should be included. It is an RCT comparing Ivermectin vs. aspirin vs. placebo. Were samples included from participants from both cohorts 1 and 2?

3. The sampling strategy of ILI surveillance (study 1) should be included; age criteria, target numbers per day/week, as number of included samples seems to vary greatly according to Figure 2.

4. Limited demographic/clinical data are included, presumably not collected by ILI surveillance. Co-morbidities and vaccination status would have been useful.

5. The manuscript highlights scarce data on respiratory viral circulation from Africa - and mentions data from South Africa (Tempia Eurosurveillance 2021) and Ntagereka (Infect Dis med Micro 2022), but should also include Loevinsohn *et al.* (2022[1]), which was published prior to this manuscript. This surveillance study in Zambia found a substantial decline in the prevalence of RSV and influenza in the first year of the pandemic, while an increase in the prevalence of rhino/enteroviruses and non-SARS-CoV-2 coronaviruses was observed after March 2020 (when SARS-CoV-2 emerged in Zambia), compared to 2019.

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Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Respiratory viral epidemiology.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 18 January 2023

https://doi.org/10.21956/gatesopenres.15460.r32800

✔ **Ivy A. Asante** [iD]

Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana

In this paper, the authors describe their observations concerning respiratory pathogens during the COVID-19 pandemic in The Gambia. The data they describe is very important especially from West Africa where such data is not readily available.

Comments:
1. From Figure 1, it looks like the authors looked at some selected sites in The Gambia, as opposed to the whole country. Even if sites were few and distributed throughout the country, it would be said to represent the country. For consideration, the authors could include 'selected sites in The Gambia' in their title.

2. The authors used a multiplex RT-PCR assay that detected a panel of respiratory pathogens. This detection however did not distinguish or sub-type viruses seen. E.g. for influenza B, lineage determination was not reported, same for influenza A and the human coronaviruses. If this data is available, it should be included.

3. Specify what UTM was used. What is the volume of the Universal transport medium? Why did the researchers choose to collect only NP swabs and not a combined OP/NP swab?

4. For the ILI surveillance system, is there a systematic approach to sample collection? E.g. A specific number expected each week? This could explain the number of samples tested during each of the months; what accounted for the high number of samples in one month and a low number in another?

5. Is the SARS-CoV-2 data observed in this study comparable to national data? It is not very clear.

6. Was there any correlation between detection of SARS-CoV-2 and other respiratory viruses?

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Virology, surveillance for respiratory viruses

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.