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Original Article

Seasonal variation of asymptomatic viral and bacterial nasopharyngeal carriage in rural Senegal

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ABSTRACT

Background: The surveillance of respiratory pathogens in rural areas of West Africa has, to date, largely been focussed on symptoms. In this prospective study conducted prior to the COVID-19 pandemic, we aimed to assess the asymptomatic prevalence of respiratory pathogen carriage in a group of individuals living in a rural area of Senegalese.

Methods: Longitudinal follow up was performed through monthly nasopharyngeal swabbing during the dry season and weekly swabbing during the rainy season. We enrolled 15 individuals from the village of Ndiop. A total of 368 nasopharyngeal swabs were collected over a one-year period. We investigated the prevalence of 18 respiratory viruses and eight respiratory bacteria in different age groups using singleplex and multiplex PCR.

Results: In total, 19.56% of the samples (72/368) were positive for respiratory viruses and 13.60% of the samples (50/368) were positive for respiratory bacteria. Coronaviruses (19/72, 26.39%), adenoviruses (17/72, 23.61%), rhinoviruses (14/72, 19.44%), *Streptococcus pneumoniae* (17/50, 34%), and *Moraxella catarrhalis* (15/50, 30%) were the most frequently detected viruses. Interestingly, the carriage of respiratory pathogens was shown to be more frequent during the rainy season, as pluviometry was shown to be positively associated with the occurrence of respiratory viruses such as influenza (P = .0078, r^2 =.523) and RSV (P = .0055, r^2 =.554).

Conclusions: Our results show a non-negligible circulation of respiratory pathogens in a rural area in Senegal (West Africa) with an underestimated proportion of asymptomatic individuals. This study highlights the fact that the circulation of viruses and bacteria in the community has been overlooked.

Introduction

The circulation of respiratory viruses is very common in human populations, causing high levels of morbidity and mortality around the world (WHO, 2017). All age groups can be affected by these infections. Every year, numerous cases of illness and death due to these infections are recorded worldwide [1]. Some of the most common bacterial organisms associated with these infections are *Streptococcus pneumoniae, Haemophilus influenzae, Staphylococcus aureus,* and *Klebsiella pneumoniae* [2]. Most often, the surveillance of respiratory infections caused by viruses is limited to the observation of clinical symptoms [3]. As a result, prevalence estimates of syndromic infection rates do not take into consideration asymptomatic or slightly symptomatic groups within the population [1,4]. One prospective study aimed at monitoring respiratory viruses in families found that 47% of respiratory infections in adults were asymptomatic [5]. In addition, asymptomatic infection rates in children could reach a prevalence of up to 52% [6]. Some studies have shown that rhinoviruses are the most commonly identified virus in asymptomatic individuals [3,7,8], with prevalence ranging from 25.9% to 32.8% [3]. It is well-known that most respiratory viruses have a seasonal distribution. However, there seems to be very little data available regarding non-epidemic seasons [9]. Asymptomatic infections are becoming an increasing concern for the

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prevention and control of respiratory diseases, although estimates of their prevalence are less documented [10].

In Senegal, there is limited information about the prevalence of respiratory viruses in asymptomatic individuals. The epidemiological data available on respiratory infections in Senegal mostly results from the surveillance of influenza and other viruses responsible for flu-like respiratory infections [11–13].

To address the lack of data on asymptomatic carriers of respiratory pathogens, frequent and recurrent sampling of individuals is required, something which rarely occurs in rural Senegal. For this purpose, we performed a one-year survey in the village of Ndiop (Senegal) to assess the prevalence of respiratory viruses and bacteria commonly detected in the population and to characterise the seasonality of respiratory viruses, while taking meteorological parameters into account.

Materials and methods

Study design

A longitudinal prospective cohort study was conducted prior to the COVID-19 pandemic between March 2019 and February 2020 in the village of Ndiop (13°41′08.01″N, 16°23′01.01″W) [14]. This village is located in the Fatick region in the centre of Senegal and had a population of 510 in 2017. Ndiop is populated by farmers and ranchers. Commercial activities are developing and transport has also been revolutionised by the adoption of Jakarta motorcycles [15]. Drinking water is mainly drawn from underground sources, despite the existence of fountains [16]. Fifteen individuals were randomly selected for this project, a cohort which included both children and adults. These individuals were asymptomatic at the time of inclusion and underwent sampling regardless of symptom presentation, weekly during the rainy season and monthly the rest of the year.

Ethics statement

Each person was invited to participate on a voluntary basis. All adult participants as well as the parents or legal guardians of participants under 18 years of age provided signed informed consent. The Senegal National Ethics Committee for Health Research (CNERS) approved this study under approval number SEN18/30.

Data collection

A standardised questionnaire was administered by the field investigators to everyone enrolled in the study. To respect the anonymity of these individuals, ID numbers were assigned to each individual and the analysis was conducted using only these numbers for identification. Data regarding demographic characteristics (age and sex) (Table S1) as well as clinical events including respiratory symptoms, chronic medical illness and influenza vaccination status were collected.

Sample collection

Randomised individuals were sampled to test for several respiratory pathogens. Nasopharyngeal swabbing was conducted between March 2019 and February 2020 on all participants. Samples were collected on at least a monthly basis and as often as on a weekly basis during critical periods, i.e. the rainy season. Weekly sampling began in October and was maintained for two months after the end of the rainy season. Inclusion kits for study participants consisted of information notices and non-opposition forms, swabs containing transport medium (Sigma Virocult® and Transwab®), and questionnaires to compile clinical and epidemiological information. Informed consent forms were obtained prior to sample collection and were kept available for the field investigators. At the beginning of each month during the dry season and every week during the rainy season, two investigators visited all participants to record their

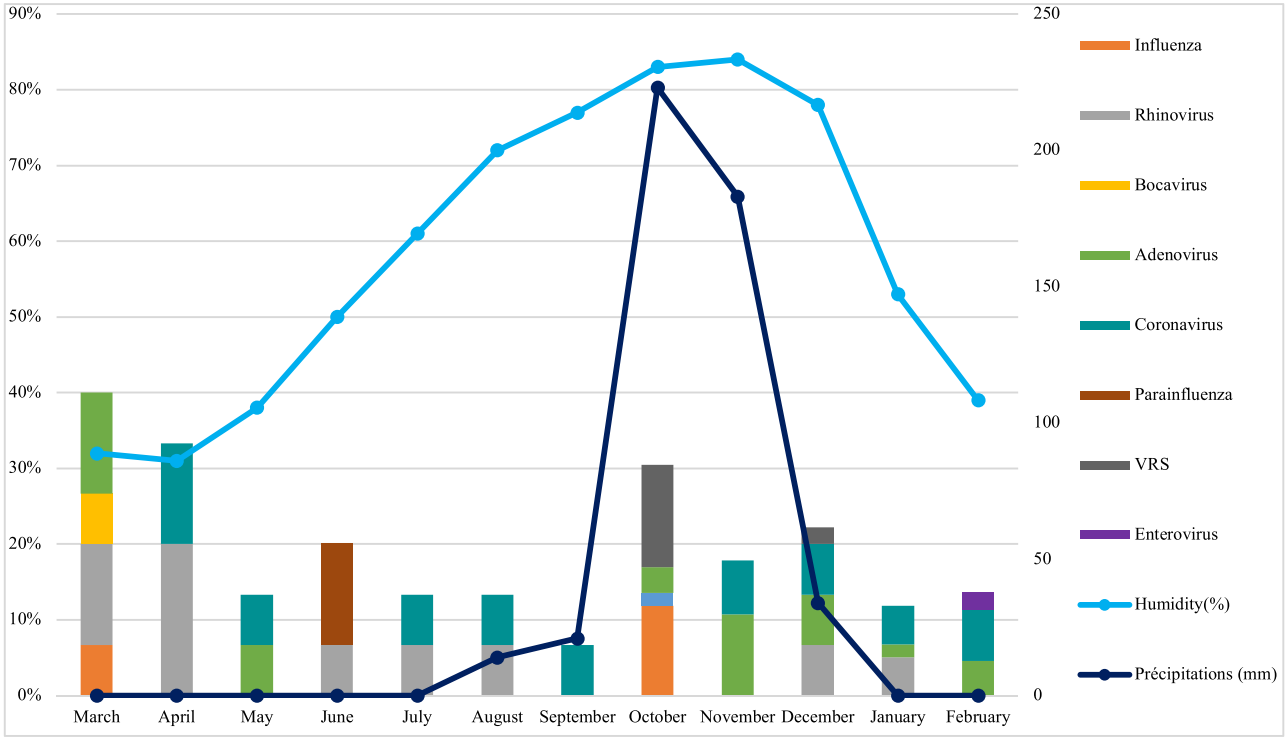

Fig. 1. Temporal distribution of positive cases of circulating respiratory viruses detected in the cohort between March 2019 and February 2020. This figure shows the prevevalence (%) of positive samples in all samples collected by month among the 15 individuals sampled during the rainy season and dry season. The right y-axis shows relative humidity (%) and the left y-axis shows rainfall (mm).

Table 1
Detection of respiratory viruses and bacteria over a one-year period and co-infection cases found in this study.

PCR test result	Monthly sampling % (n)							Weekly sampling % (n)				
	Mar-19	Apr-19	May-19	Jun-19	Jul-19	Aug-19	Sept-19	Oct-19	Nov-19	Dec-19	Jan-20	Feb-20
Positive for respiratory virus	40 (6/15)	33.3 (5/15)	13.3 (2/15)	20 (3/15)	13.3 (2/15)	13.3 (2/15)	0 (1/15)	30.5(18/59)	17.9(10/56)	22.2(10/45)	11.9 (7/59)	13.6 (6/44)
Positive for respiratory bacteria	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	13.3 (2/15)	0(3/15)	32 (16/59)	17.9(10/56)	22.2(10/45)	6.7 (4/59)	0 (0/44)
Co-infection virus-virus	6.7 (1/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	13.3 (2/15)	0 (0/15)	0 (0/59)	1.8 (1/56)	0 (0/45)	1.7 (1/59)	2.3 (1/44)
Co-infection bacteria-bacteria	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	1.7 (1/59)	1.8 (1/56)	2.2 (1/45)	1.7 (1/59)	0 (0/44)
Co-infection bacteria-virus	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	0 (0/15)	8.5 (5/59)	5.3 (3/56)	4.4(2/45)	0 (0/59)	0 (0/44)

This table shows the number of cases detected by month among all participants and/or those who tested positive during the rainy season and dry season.

body temperature and any symptoms of respiratory infection, or any other symptom listed on the clinical information questionnaire. Samples were collected regardless of the presence or absence of respiratory symptoms, using commercial rigid cotton-tipped swab applicators (Medical Wire & Equipment, Wiltshire, UK) which were inserted into the anterior nose and then placed in the viral transport medium. Swabs were kept at 4 °C prior to shipping to the "Vecteurs – Infections Tropicales et Méditerranéennes" (VITROME) Dakar laboratory for storage at − 80 °C within 48 h of collection and subsequently transported on dry ice to Marseille, France, for molecular analysis.

Nucleic acid extraction

To extract RNA/DNA from respiratory samples, the EZ1 Advanced XL (Qiagen, Hilden, Germany) was used with the Virus Mini Kit v2.0 (Qiagen), following the manufacturer's recommendations [17,18]. In a tube containing 200 µl of each sample, we added 200 µl of AVL and 4 µl of internal control for extraction in a final elution volume of 50 µl.

Identification of respiratory pathogens by virological tests

Identification of respiratory viruses

Real-time PCR was performed using the LightCycler® 480. The Fast Track Diagnostics Respiratory pathogen 21, a quantitative in vitro amplification test, allowed viral nucleic acids of specific viruses from nasopharyngeal swabs to be detected concomitantly, according to the manufacturer's recommendations. This multiplex test detects influenza virus A (IAV), influenza virus A H1N1 of swine origin (IAV [H1N1] swl), influenza virus B (IBV), rhinovirus (RV), coronaviruses (CoV 229E, NL63, HKU1 and OC43), parainfluenza viruses (PIV) 1 to 4, metapneumovirus (MPV), bocavirus (BoV), respiratory syncytial virus (RSV), parechovirus (PeV), enterovirus (EV), and adenovirus (ADV). For respiratory viruses, positive amplification results were defined as a threshold cycle (Ct) ≤ 35. An internal control (equine arteritis virus), a negative control, and a positive control provided with the FTD kits were included in each assay.

Identification of respiratory bacteria

Nasal swabs were also screened for *H. influenzae*, *K. pneumoniae*, *S. aureus*, *S. pneumoniae*, *Streptococcus pyogenes, Mycoplasma pneumoniae, Moraxella catarrhalis* and *Corynebacterium propinquum* using real-time PCR with the LightCycler® 480 and the Probes Master kit (Roche Diagnostics, France) according to the manufacturer's recommendations. The target genes for these pathogens are OMPp1, phoE, nucA, lytA, hpt, and cop B, respectively. We considered samples to be positive if the qPCR were positive with the cycle number at the threshold level of log-based fluorescence Ct value ≤ 35 [19].

Environmental data

Data regarding environmental factors such as monthly rainfall (mm), average minimum and maximum temperature (°C) and relative air humidity (%) were acquired from the VITROME laboratory and the National Agency of Civil Aviation and Meteorology of Senegal (ANACIM) for the 2019–2020 study period.

Statistical analysis

The prevalence of pathogens was calculated by dividing the total number of positive cases by the total number of samples collected in the dry season and rainy season. We performed a factor analysis to visualise correlations between respiratory pathogens and the seasons. Correlation between viruses and pluviometry, and viruses and relative humidity was performed using GraphPad Prism version 8.0.0

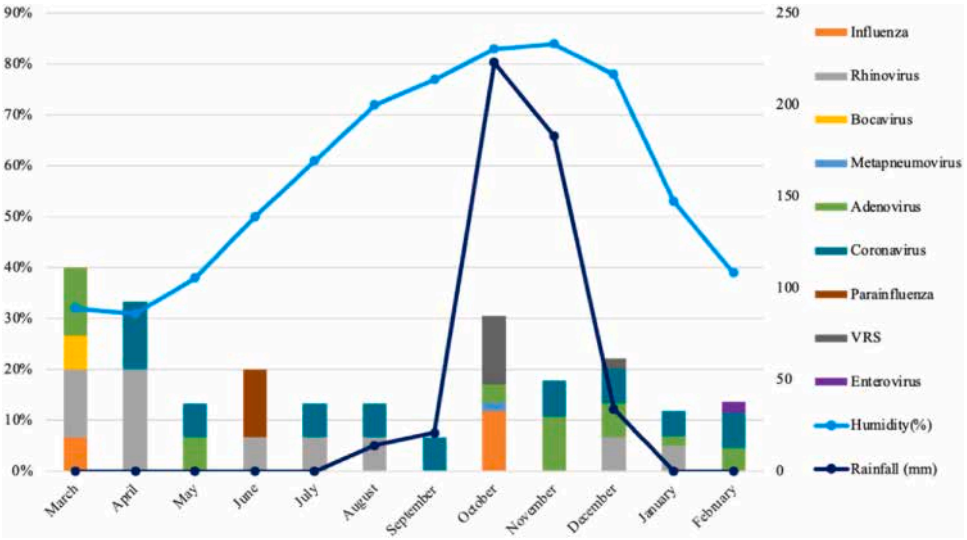

Fig. 2. Detected bacterial pathogens by qPCR in respiratory samples of the cohort between March 2019 and February 2020. This figure shows the prevevalence (%) of all positive samples collected by month from the 15 individuals sampled during the rainy season and dry season.

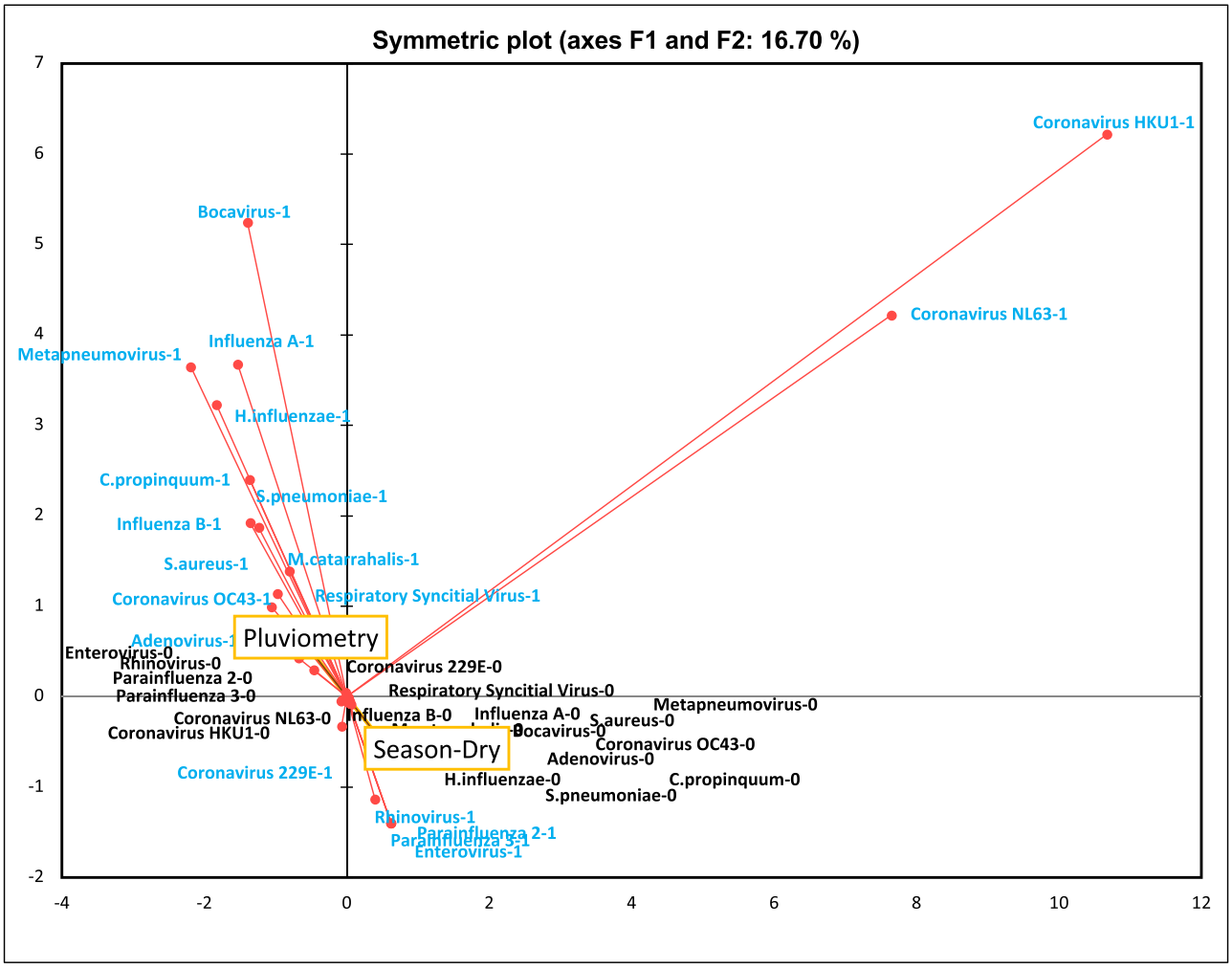

Fig. 3. Factor analysis between respiratory pathogens and seasons.

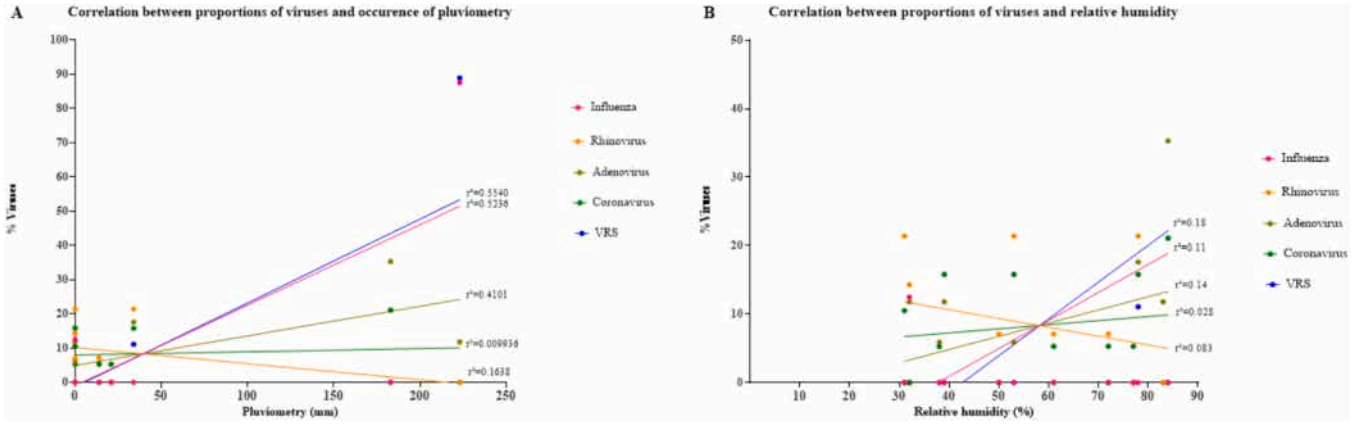

Fig. 4. Correlation of viruses with pluviometry (A) and relative humidity (B). Differences were considered to be statistically significant if the r^2 value > 0.5, indicating a correlation between some respiratory viruses, here influenza, rhinovirus and adenoviruses, and the increased rainfall.

for Windows, GraphPad Software, San Diego, California, USA, www.graphpad.com. All other statistical analyses were performed using the R software environment for statistical computing (V4.2.3). Factor analysis was performed using the FactoMineR package [20]. The level of significance for all the statistical tests was set at $\alpha = .05$.

Results

Characteristics of the study population

This study consisted of 15 individuals consisting of three children and 12 adults. The median age was 29 years (ranging from 11 to 76 years) and three individuals (23%) were males (Table S1). A total of 368 nasopharyngeal swabs were collected. Four participants presented the following chronic diseases: hypertension, osteoarthritis, chronic dermatosis, and asthma, and two participants were suffering from severe anaemia (Table S1). None of the participants reported being vaccinated against influenza viruses. Pathogen carriers were identified among participants over the entire study period. However, positivity levels were lower in May, June, July, and August, prior to the rainy season, than in the rest of the year. A peak in October was concomitant with the maximum rainfall peak (223 mm) (Fig. S1).

Viral carriage of respiratory samples

Some 19.56% of the samples (72/368) were identified by qPCR and were positive for at least one of the 18 respiratory viruses tested. Several circulation patterns were observed for the tested viruses. The most prevalent micro-organisms detected were coronaviruses, rhinoviruses and adenoviruses. Coronaviruses (229E, NL63, HKU1, and OC43) were detected during nine of the 12 months, with a prevalence among positives samples of 26.39% (19/72). No coronaviruses were detected in March, June, or October. Rhinoviruses (19.44%, 14/72) and adenoviruses (23.61%, 17/72) were detected in seven of the 12 months (Fig. 1). The circulation pattern of adenoviruses reflected seasonality between October and March with greater activity in November. Peaks of RSV (12.50%, 9/72) and influenza (11.11%, 8/72) appeared simultaneously in October, when relative humidity and rainfall were at their highest level (Fig. 1). A lower prevalence was observed for bocavirus (1.38%, 1/72), metapneumovirus (1.38%, 1/72), enterovirus (1.38%, 1/72) and parainfluenza virus (2.78%, 2/72). The prevalence of respiratory virus carriage among these participants was higher in adults and ranged from 6.70% to 60%. The annual infection rate in these individuals ranged from one to nine (Fig. S2). Co-infection cases were detected in four participants during the study period (Table 1) including one case of influenza A and bocavirus co-infection in March, one case of adenovirus and coronavirus OC43 in November, and two cases of coronavirus HKU1 and coronavirus NL63 co-infection in January and February in two different participants.

Bacterial diagnosis of respiratory samples

Regarding respiratory bacterial carriage, 13.60% of samples (50/368) were positive by qPCR (Table 1). *Streptococcus pneumoniae* was most often detected with a prevalence of 34.0% (17/50), followed by *M. catarrhalis* (30.0%, 15/50), *S. aureus* (16.0%, 8/50), *H. influenzae* (12.0%, 6/50), and *C. propinquum* (8.0%, 4/50). *Klebsiella pneumoniae, S. pyogenes* and *M. pneumoniae* were not detected. During the one-year study, respiratory bacteria were only detected in the period between September 2019 and January 2020 (Fig. 2). Co-infections were observed involving *S. pneumoniae* and *H. influenzae* in two cases, *H. influenzae* and *S. aureus* in one case, and finally *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* in another case (Table 1).

Correlation between pathogens and environmental factors

The seasonal patterns of the respiratory pathogens were studied. Overall, carriage of respiratory bacteria was more frequent during the rainy season than the dry season (Figs. 2 and 3). Seasonal variations were observed for RSV ($P < .0005$), *H. influenzae* ($P < .0461$), *S. pneumoniae* ($P < .0018$), *M. catarrhalis* ($P < .0036$) and *C. propinquum* ($P < .0350$) (Tables S2 and S3).

For the most frequently detected viruses (coronaviruses, adenoviruses, rhinoviruses, RSV, and influenza), we also sought a correlation with pluviometry and relative humidity. Regarding pluviometry, no significant difference was noted between overall carriage in the dry season and in the rainy season ($P = .7061$). However, a positive and significant correlation was obtained with influenza ($P = .0078$, $r^2 = .5236$; Fig. 4, Table S4), and RSV ($P = .0055$, $r^2 = .5540$; Fig. 4, Table S2). In addition, there was a lower correlation between adenovirus ($P = .0249$, $r^2 = .4101$, Fig. 4, Table S5) and rainfall. Conversely, no correlation was observed between rainfall and coronavirus and rhinovirus.

Interestingly, relative humidity did not seem to be associated with the incidence of these respiratory viruses, as there was no correlation between relative humidity and respiratory viruses (Fig. 4, Table S6).

Strong correlations were also found when creating a correlation matrix (pathogens versus pathogens) (Table S2) for coronaviruses HKU1 and NL63 ($P < .0001$), *M. catarrhalis* and coronavirus OC43 ($P < .0089$), metapneumovirus and *S. pneumoniae* ($P < .0435$), influenza A and bocavirus ($P < .013$), and *H. influenzae* and *S. pneumoniae* ($P < .0021$).

Discussion

Respiratory infections are highly transmissible, and the circulation of respiratory pathogens is omnipresent. However, very limited data are available on the prevalence of asymptomatic infections in the global population. Most studies in Africa are either based on syndromic surveys [11,21–23] or are focused on risk groups [12,24–28].

During our twelve-month longitudinal study, we highlighted non-negligible coronavirus carriage rates in asymptomatic individuals, with a major viral detection rate for adenovirus (23.61%), rhinovirus (19.44%), *S. pneumoniae* (34%) and *M. catarrhalis* (30%), in line with other studies [3,29]. *Streptococcus pneumoniae* has also previously been found to have a high detection rate in the same area (Dielmo and Ndiop) in one study on skin carriage [16].

Another recent study also reported a high level of asymptomatic carriage of rhinovirus and coronavirus among an adult outpatient population in New York city [1]. Other studies carried out in the USA have reported overall asymptomatic carriage of 11% among healthcare workers [30,31]. Indeed, the RNA of some viruses, such as rhinovirus for instance, can be detected for several weeks following an infection. In addition, shedding also occurs prior to the development of symptoms [3]. This may explain the high frequency of detection of asymptomatic coronaviruses, adenovirus and rhinovirus RNA, as observed in other studies in Senegal [23] and other countries [5,32]. The asymptomatic carriage of *M. catarrhalis* was also found to be high (30%), as observed in the context of a mass gathering in Senegal (21%). Involving around 4–5 million individuals from different places, this gathering led to crowded settings, known to increase the transmission of respiratory pathogens [22]. Close-contact environments are favourable for the transmission of respiratory pathogens. Similarly, in Ndiop, the spread of respiratory pathogens seems to be linked to the overcrowded living conditions associated with rural lifestyles. Ndiop is a village of farmers and ranchers and their living quarters often involve close proximity among individuals as well as close contact between the population and their livestock, a well-known pathogen reservoir.

Environmental factors such as rainfall and relative humidity have been reported to increase viral survival in the environment and may increase the risk of indirect virus transmission [33]. In our study, a prevalence peak was recorded for RSV, influenza and adenoviruses during the rainy season. Regarding influenza activity, only one significant peak during the rainy season was observed, in line with other observations reported in tropical regions such as Cambodia, Thailand [34] and Côte d'Ivoire [35], where a high incidence was noted during this period [36]. Another study conducted in Senegal also showed similar detection rates and circulation patterns for RSV [37]. In contrast, relative humidity does not show any definite correlation with these viruses. However, we can clearly see that influenza and RSV appear when relative humidity is higher, as previously reported by Price and colleagues [36].

This study highlights a variety of respiratory viruses in individuals of all ages, and indicates a clear pattern of circulation of these respiratory viruses. Our study has limitations, particularly the small number of samples included and the fact that weekly sampling only took place during the rainy season. However, the non-stringent inclusion criteria made it possible to detect asymptomatic carriers. Some viruses, such as influenza, are over-represented among patients requiring treatment, while others, such as coronaviruses, are deeply under-represented and can easily be spread through asymptomatic and/or mildly symptomatic infected individuals. Although conducted with a small sample size, this pilot study establishes the feasibility and efficiency of our data and sample collection methods and highlighted potential challenges for future large-scale studies. Indeed, in light of the non-syndromic surveillance data presented here, a wider scale study should be conducted to increase the number of household surveys in order to clarify the actual prevalence of respiratory virus infections within the rural population.

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Declaration of Competing Interest

The authors have no competing interests to report.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jiph.2024.03.020.

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