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RESEARCH ARTICLE

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Epidemiology and genetic characterization of respiratory syncytial virus in children with acute respiratory infections: Findings from the influenza sentinel surveillance network in Central African Republic, 2015 to 2018

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Abstract

Background and aims: Respiratory syncytial virus (RSV) is one of the main viral pathogens causing acute respiratory infections in children under 5 years of age but has seldom been studied in Central African Republic (CAF). Taking advantage of the national influenza surveillance network in CAF, this study aimed at providing the first insights into RSV prevalence and seasonality over 4 years of surveillance and the clinical manifestations of RSV in this population in CAF.

Methods: A total of 3903 children under 5 years matching the influenza-like illness (ILI, 68.5%) or severe acute respiratory infection (SARI, 31.5%) case definitions were recruited from January 2015 to December 2018. The presence of RSV viral RNA in nasopharyngeal samples was assessed by RT-PCR, followed by RSV-A and RSV-B typing and Sanger sequencing on a subset of samples. Phylogenetic analyses were carried on partial G protein sequences. Associations between RSV and demographic or clinical manifestations were investigated by statistical analyses.

Results: RSV prevalence was significantly higher in infants <6 months (13.4%), in hospitalized children (13.3% vs 5.5%) and in male patients (9.5% vs 6.4%). An overall prevalence of RSV of 8.0% in the period of 2015 to 2018 was shown, with significant annual (6.4%-10.6%) and seasonal (12.7% in rainy season vs 3.0% in dry season) fluctuations. While RSV seasons in 2015, 2016, and 2018 were relatively similar, 2017 showed deviations from the overall patterns with significantly higher RSV circulation and an outbreak peak 3 to 5 months earlier. Concomitant circulation of RSV-A and RSV-B with an alternating predominance of RSV-A and RSV-B strains and temporal RSV-A genotype replacement from NA1 to ON1 was observed.

had no role in the study design, collection, data analysis, and interpretation or manuscript writing

Conclusion: This study represents the first in-depth epidemiological analysis of RSV in CAF and provides first insights into RSV genetic diversity and seasonality in the country.

KEYWORDS
Central African Republic, children, epidemiology, genotype, respiratory syncytial virus, seasonality

1 | INTRODUCTION

Respiratory syncytial virus (RSV), first characterized in 1956,[1] often causes lower respiratory tract infections in infants. It is the second leading cause of bronchiolitis and pneumonia, particularly during the winter months in temperate countries and during the rainy season in tropical countries.[2-6] RSV was estimated to be responsible for more than 90 million deaths worldwide between 1995 and 2010, including 20 million deaths in sub-Saharan Africa alone.[7,8]

RSV belongs to the order *Mononegavirales*, the family *Pneumoviridae*, the genus *Orthopneumovirus* and the species *Human orthopneumovirus*. It is an enveloped virus, with a linear, negative-sense RNA genome of about 15 000 base pairs (bp). The genome contains 10 genes that code for 11 proteins.[5] Among them, the surface glycoproteins G and F are the main targets of neutralizing antibodies.[9] The F protein governs the fusion of the viral envelope with the cell membrane. The glycoprotein G is involved in the attachment of the virus to the CX3CR1 chemokine receptor expressed on epithelial cells and facilitates the penetration of the virus into the host cell.[10] The variability of the G protein facilitates evasion of the immune response, allowing reinfections throughout life and complicates vaccine development.[11] Epidemiological and molecular studies on the antigenic and genetic variability of the G protein have classified RSV into two highly divergent phylogenetic sub-groups A and B.[3,12,13] To date, 15 RSV-A genotypes and 37 RSV-B genotypes have been defined.[14-17] In the RSV-A subgroup, the ON1 genotype which contains a 72 nucleotide (nt) duplication in the G protein,[2,4] was first described in 2010 in Canada and is currently the most frequent RSV-A genotype. In RSV-B, BA genotypes, first detected in 1999, have now become the predominant RSV-B circulating worldwide and carry a 60 nt duplication.[18,19]

In Central African Republic (CAF), respiratory infections represent the most common cause for seeking medical care.[20] A preliminary study reported RSV in 3.0% (10/329) of children aged 0 to 15 years enrolled from January to December 2010.[21] However, the study included a relatively small (329 patients), heterogeneous (children 0-15 years) target population recruited over a limited timespan (12 months). No strain characterization was performed. Here, we analyzed epidemiological and clinical data and characterized RSV strains from children under 5 years of age, hospitalized or not, presenting with clinical signs of respiratory infections, and recruited in the national sentinel influenza network. We provide first insights into RSV prevalence and seasonality over 4 years of surveillance and the clinical manifestations of RSV in this population in CAF.

2 | METHODS

2.1 | Sentinel influenza surveillance network in CAF, case definition, and study population

This study was carried out as part of the national influenza surveillance programme in CAF. The surveillance program started in 2008[20] and currently includes five sentinel sites selected based on total number of consultation per year and accessibility for sample transport. Two sites are located in the capital Bangui: the tertiary pediatric referral hospital pediatric complex (284 beds) and the Saint Joseph hospital (8 beds). The surveillance also includes the hospitals of the cities Boali (5 beds) and Bossembolé (27 beds) located 90 and 180 km North-East of Bangui, as well as the healthcare centre of Pissa (6 beds) located 70 km South-West of Bangui.[20] Except for the pediatric hospital, the only such structure in the country, which receives children from 0 to 15 years old, all other sentinel sites treat patients of any age and consulting for any reason. The total number of pediatric consultations (children <5 years old) for any cause per site per year in all sentinel sites for the 2015 to 2018 surveillance period is depicted in Figure S1.

Within the sentinel surveillance, the WHO criteria for ILI (measured fever or history of fever ≥38°C and cough with onset within the last 10 days) or SARI (measured fever or history of fever ≥38°C and cough with onset within the last 10 days requiring hospitalization)[22] were adopted. The study population included outpatients and inpatients under the age 5 who met the ILI and SARI case definitions, recruited from January 2015 to December 2018 at the five sentinel sites after obtaining consent.

2.2 | Sample and data collection

For each participant, a nasopharyngeal swab was collected then placed in a labeled tube with 3 mL of Universal Transport Medium (Copan, Italy). Samples were stored at 4°C or sent immediately in a refrigerated box to the National Reference Centre for Influenza for diagnosis. Each sample was then aliquoted into four 1.5 mL Eppendorf

tubes for influenza virus culture, extraction of nucleic acids, and long-term storage at −80°C.

Social, demographic, clinical, and epidemiological data were recorded for each patient using a standardized questionnaire. The total number of consultations per site per year for any cause as well as for ILI symptoms were retrieved from the national database hosted at the Institut Pasteur de Bangui. This database is populated by standardized reports provided weekly by the sentinel sites.

2.3 | Nucleic acid extraction and RSV detection by RT-PCR

Within 24 hours after sample reception, RNA was extracted from a 140 μL nasopharyngeal swab sample aliquot using the QIAamp Viral RNA Mini kit (Qiagen, USA) according to the manufacturer's instructions. RSV was detected by a conventional multiplex one-step RT-PCR detecting human metapneumovirus, influenza A, and influenza B virus as described before.[23] Cycling conditions were as follows: 50°C for 30 minutes, 94°C for 15 minutes followed by 40 cycles of 94°C for 30 seconds, 55°C for 30 seconds, 72°C for 1 minute, and a final extension at 72°C for 10 minutes.

2.4 | RSV typing and sequencing

RSV strain characterization was performed in a second step, using a batch approach, when sufficient number of samples were accumulated. RSV positive samples were typed as RSV-A or RSV-B by targeting fusion protein F by a semi-nested PCR as described before.[24] cDNA synthesis was carried out using the SuperScript III First-Strand enzyme (Invitrogen, USA) and the antisense primer F164 (5′-GTTATGACACTGGTATACCAACC-3′).[24]

The first round amplification was carried out with 5 U/μL Taq DNA polymerase (Promega, USA) and 10 μM of each primer, ABG490 (5′-ATGATTWYCYTTTGAAGTGTTC-3′) and F164. Two semi-nested PCRs were then performed on each sample with the AG655 (5′-GATCYCAAACCTCAAACCAC-3′) or BG517 (5′-TTYGTTCCCTGTA GTATATGTG-3′) sense primers and F164 antisense primer.[24]

PCR products were separated by electrophoresis on a 1.5% agarose gel. Samples with a 450 bp band in the first semi-nested PCR were typed as RSV-A, and samples with a band between 585 and 645 bp in the second semi-nested PCR were typed as RSV-B. These amplicons were then purified using the QIAquick PCR Purification kit (Qiagen, Venlo, the Netherlands) according to the manufacturer's protocol. Purified products were sequenced using the corresponding PCR primers on an ABI 3130 capillary sequencer.

2.5 | Phylogenetic analysis

Consensus sequences were generated using SeqScape software (version 2.5) and analyzed with BLAST (http://www.ncbi.nlm.nih.gov.BLAST/) for similarity searches. All sequences were aligned using ClustalW implemented in BioEdit v7.2.5.[25] Evolutionary distances were calculated using the Maximum Composite Likelihood model and are expressed as the number of nucleotide substitutions per site. The best nucleotide substitution model was selected with MEGA v6.06,[26] and used to calculate the phylogenetic trees with the maximum likelihood method with 1000 bootstrap iterations as implemented in MEGA v6.06.

2.6 | Amino-acid analysis

The nucleotide sequences were translated into amino-acid sequences using the standard genetic code implemented in BioEdit software. The amino-acid sequences were compared with those from the prototype RSV-A (ON1: JN257693, Canada; NA1: AB470478, Canada) and RSV-B strains (BA9: AY333364, Argentina). BioEdit was used to visualize the amino-acid variability in the second hypervariable part of the G protein.

2.7 | Statistical analyses

Statistical analyses were performed in SigmaPlot v12.5, using Mann-Whitney rank sum tests for continuous variables, χ^2-test for categorical variables or *z*-test for low proportions. Odds ratios were calculated with χ^2-test with the Yates correction.

A modified clinical severity score based on the scale from Pierangeli et al[27] was calculated for all SARI patients for whom oxygen saturation levels (>95% = 0, 95%-90% = 1, <90% = 2), respiratory rate (<45 breaths/min = 0, 45-60 breaths/min = 1, >60 breaths/min = 2), chest indrawing (absent = 0, present = 1), and feeding ability (normal = 0, reduced = 1) were measured (n = 358). Clinical severity was then stratified into two groups: moderate (scores 0-3) and severe (scores 4-6).[27] Logistic regression analysis was performed using R software (version 4.0.4)[28] with "tidyverse,"[29] "MASS,"[30] "epitools,"[31] "car,"[32] and "readxl"[33] packages to assess the association of clinical severity (included as a binomial response variable) with patient's age group, sex, and RSV status.

3 | RESULTS

3.1 | Participant enrollment

From January 2015 to December 2018, a total of 112 214 children below 5 years old attended the five sentinel sites, all causes included (Figure S1). Among them, 6238 (5.6%) children presented with respiratory symptoms characteristic of ILI. A total of 2672 (42.8%) of all ILI cases were enrolled in the study. Despite some fluctuations in the recruitment rate between sites and between years, the annual ILI recruitment rate across all sites was rather constant (range: 39.8%-46.9%; Figure S2). Only few ILI cases were recruited at the Paediatric Complex. Overall, 1231 children presenting with a respiratory syndrome corresponding to the SARI (31.5% of all recruited participants) case definitions were included in the study. SARI cases were mainly

TABLE 1 Demographic characteristics of patients included in the study, by year

Demographic characteristics Total number	2015 (N = 813)	2016 (N = 968)	2017 (N = 1084)	2018 (N = 1038)	Total (N = 3903)
Sex, N (%)					
Male	434 (53.4)	466 (48.1)	592 (54.6)	534 (51.4)	2026 (51.9)
Female	379 (46.6)	502 (51.9)	492 (45.4)	504 (48.6)	1877 (48.1)
Age, N (%)					
0 to 6 months	253 (31.1)	261 (27.0)	318 (29.3)	226 (21.8)	1058 (27.1)
7 to 12 months	130 (16.0)	150 (15.5)	177 (16.3)	206 (19.8)	663 (17.0)
13 to 24 months	157 (19.3)	216 (22.3)	239 (22.0)	218 (21.0)	830 (21.3)
25 to 36 months	128 (15.7)	154 (15.9)	170 (15.7)	194 (18.7)	646 (16.6)
37 to 48 months	81 (10.0)	100 (10.3)	91 (8.4)	107 (10.3)	379 (9.7)
49 to 60 months	64 (7.9)	87 (9.0)	89 (8.2)	87 (8.4)	327 (8.4)
Sites, N (%)					
Boali	129 (15.9)	212 (21.9)	314 (29.0)	224 (21.6)	879 (22.5)
Bossembélé	149 (18.3)	173 (17.9)	93 (8.6)	164 (15.8)	579 (14.8)
Paediatric complex	75 (9.2)	217 (22.4)	348 (32.1)	180 (17.3)	820 (21.0)
Pissa	251 (30.9)	237 (24.5)	242 (22.3)	235 (22.6)	965 (24.7)
Saint Joseph	209 (25.7)	129 (13.3)	87 (8.0)	235 (22.6)	660 (16.9)
Clinical case, N (%)					
ILI	630 (77.5)	623 (64.4)	606 (55.9)	813 (78.3)	2672 (68.5)
SARI	183 (22.5)	345 (35.6)	478 (44.1)	225 (21.7)	1231 (31.5)

recruited (56.1%) at the Paediatric Complex, while between 3.8% and 16.5% of SARI cases were recruited in the other four centers. Unfortunately, no data on the overall number of SARI cases per site per year were available.

3.2 | Demographic and clinical characteristics of patients

The demographic and clinical characteristics for all enrolled patients are shown in Table 1. Slightly more nasopharyngeal samples were collected from male (51.9%) than female patients (48.1%; female:male ratio of 0.9:1). There were little differences in the age and sex distribution across the 4 years of the study, but there was some variability between study sites. Children aged between 0 and 6 months had an increased risk of presenting with more severe symptoms necessitating hospitalization compared to older children ($P < .001$), while females had a lower risk of presenting with SARI compared to male (OR = 0.84, $P = .014$; Table S1).

3.3 | RSV detection and association with age and sex

In total, 8.0% (312/3903) of all patients were tested positive by RT-PCR for the presence of RSV (Table 2). Increased RSV detection in males (9.5%) compared to females (6.4%) was observed (OR = 1.53, $P < .001$; Table 3). Children aged 0 to 6 months (13.4%) had an increased risk to be RSV-positive compared to all other age groups (4.0%-7.5%; $P < .001$; Table 3).

3.4 | Disease severity and RSV-related deaths

RSV-positive cases were more frequently detected among hospitalized patients compared to outpatients (13.3% vs 5.5%, OR = 2.62, $P < .001$; Table 3). RSV was associated with dyspnea ($P < .001$), wheezing ($P < .001$), chest indrawing ($P < .001$), and inability to feed ($P = .002$) but not with rhinorrhoea ($P = .993$), diarrhoea ($P = .983$), vomiting ($P = .107$), lethargy ($P = .816$), or convulsion ($P = .752$; Table S2). In hospitalized children, RSV was significantly more frequently detected in children with a diagnosis of bronchiolitis or bronchopneumonia compared to pneumonia (Table 3) and was associated with increased duration of hospitalization ($P < .001$). Among SARI patients for whom clinical data were exhaustive (n = 358), only younger age was significantly associated with increased odds of showing higher clinical scores, while sex and RSV status were not significantly associated (Table S3). In an alternative statistical model with clinical scores stratified as moderate (scores 0-2) and severe (3-6), RSV-positive children had increased risk of experiencing a more severe disease (OR = 1.93; 95% CI: 1.10-3.39; $P = .022$; Table S4). The difference between the two models likely arose from the limited number of patients included and the high proportion of RSV-positive children with intermediate clinical scores (score = 3).

TABLE 2 RSV prevalence per year, all patients included and stratified by ILI or SARI syndrome

Year	No. of RSV-positive children/Total No. of patients enrolled (%)	*P* value	No. of RSV-positive children among ILI patients/Total No. of ILI patients enrolled (%)	*P* value	No. of RSV-positive children among SARI patients/Total No. of SARI patients enrolled (%)	*P* value
2015	52/813 (6.4)	Ref	32/630 (5.1)	Ref	20/183 (10.9)	Ref
2016	64/968 (6.6)	.931	32/623 (5.1)	.934	32/345 (9.3)	.650
2017	115/1084 (10.6)	.002	31/606 (5.1)	.920	84/478 (17.6)	.048
2018	81/1038 (7.8)	.283	53/813 (6.5)	.299	28/225 (12.4)	.750
Total	312/3903 (8.0)		148/2672 (5.5)		164/1231 (13.3)	

TABLE 3 Demographic and clinical characteristics associated with increased odds of RSV detection

Variable	No. of patients tested positive for RSV/Total No. of patients (%)	OR (95% CI)	*P* value	Proportion of all RSV positive patients (%; n = 312)
Sex				
F	120/1877 (6.4)	Ref		38.5
M	192/2026 (9.5)	1.53 (1.21-1.94)	*P* < .001	61.5
Age group				
0 to 6 months	142/1058 (13.4)	Ref		45.5
7 to 12 months	50/663 (7.5)	0.53 (0.38-0.74)	*P* < .001	16.0
13 to 24 months	50/830 (6.0)	0.41 (0.30-0.58)	*P* < .001	16.0
25 to 36 months	36/646 (5.6)	0.38 (0.26-0.56)	*P* < .001	11.5
37 to 48 months	21/379 (5.5)	0.38 (0.24-0.61)	*P* < .001	6.7
49 to 60 months	13/327 (4.0)	0.27 (0.15-0.48)	*P* < .001	4.2
Clinical case				
ILI	148/2672 (5.5)	Ref		47.4
SARI	164/1231 (13.3)	2.62 (2.08-3.31)	*P* < .001	52.6
Diagnosis upon admission[a]				
Pneumonia	45/332 (13.6)	Ref		14.4
Bronchopneumonia	14/46 (30.4)	2.79 (1.32-5.63)	*P* = .006	4.5
Bronchiolitis	63/311 (20.3)	1.62 (1.07-2.46)	*P* = .030	20.0
Not determined or others	42/542 (7.7)	-	-	13.3

[a]For hospitalized children only.

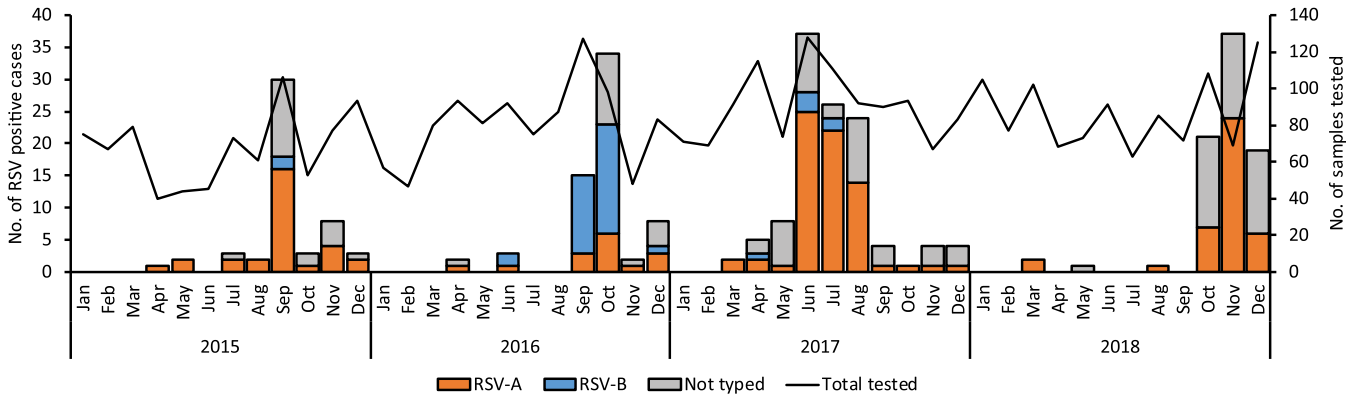

FIGURE 1 Distribution of RSV cases by month over the 4 years of the study

In total 58/3903 (1.5%) of the patients enrolled died, among which 8 (13.8%; two females, six males) had a RSV infection, but no association between RSV infection and death was observed ($P = .162$). For these eight patients, all younger than 24 months, delay between symptom onset and hospitalization ranged from 0 to 7 days, while the delay until hospitalization and death ranged from 0 to 9 days. Co-morbidities were reported for four patients and included malnutrition, malaria, and congenital heart disease. When measured (5/8), oxygen saturation levels were <95%. Bacterial co-infections with *Staphylococcus aureus*, *Hemophilus influenzae*, *Moraxella catarrhalis*, *Streptococcus pneumoniae*, and/or co-infections with influenza A virus were detected in five patients (Table S5).

3.5 | Seasonal circulation of RSV

RSV detection rates varied across years and ranged from 6.4% in 2015 to 10.6% in 2017 (Table 2). Prevalence among all cases in 2017 was significantly higher ($P = .002$). However, RSV prevalence among ILI cases did not vary between years (range: 5.1%-6.5%; Table 2), while RSV prevalence among children presenting with SARI symptoms significantly increased in 2017 ($P = .048$, Table 2).

During the study period, RSV detections started in March-April and lasted until December (Figures 1 and 2). Sporadic detections were recorded between March-April and August and RSV circulation peaked in September (2015), October (2016), or November (2018). In addition to a significantly higher number of cases reported in 2017, the peak of RSV incidence occurred in June, 3 to 5 months earlier than during the other 3 years (Figure 2). RSV was also significantly more frequently detected in the rainy season (June-November) compared to the dry season (December-May; 255/1756, 12.7% vs 57/1892, 3.0%; $P < .001$).

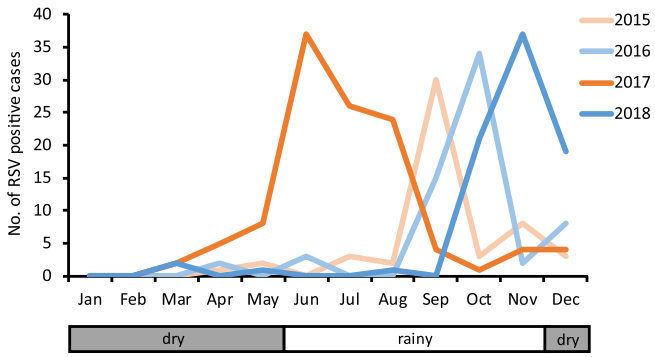

FIGURE 2 Overlap of RSV seasons over the 4 years (2015-2018) of the study and overall dry/rainy season distinction

Among RSV cases, 155 (49.7%) belonged to the RSV-A sub-group and 40 (12.8%) to the RSV-B sub-group, while 117 (37.5%) could not be typed (Table 4). Among typed RSV strains, high RSV-A circulation was observed in 2015 (30/32, 93.7%), 2017 (70/76, 92.1%), and 2018 (40/40, 100%). RSV-B was detected in 2015 to 2017 and was predominant in 2016, representing more than half of the RSV-positive cases (32/47, 68.1%; Figure 3; Table 4).

3.6 | Phylogenetic analysis

Glycoprotein gene sequencing was attempted for positive samples successfully typed as RSV-A or RSV-B and resulted in 160 partial G gene sequences. Based on phylogenetic analyses, 17 genotype RSV-A NA1, 120 genotype ON1, and 23 RSV-B genotype BA9 sequences were identified (Figure 3). RSV-A NA1 genotype was almost exclusively detected in 2015 (15/17, 88.2%), while RSV-A genotype ON1 replaced NA1 as of 2016 (Figure 3).

Genetic distances between strains of the same genotype ranged from 0% to 1.4% for RSV-A NA1, 0% to 6.3% for RSV-A ON1, and 0% to 6.4% for RSV-B BA9. Five unique NA1 sequences (5/17, 29.4%) were observed, and the same strain was found in 2015 to 2017 (-Figure S3). Twenty-six unique nucleotide sequences clustered within ON1 genotype (26/120, 21.7%). The same strains were found over 1 or 2 years on only four occasions, while a large cluster of identical strains (n = 79) was found in 2017 to 2018 which might suggest local transmission. Ten unique BA9 sequences were identified (10/23, 43.5%; Figure S4). Four unique strains were interspersed within the BA9 genotype, while the other six unique strains (corresponding to 18 sequences in total) formed a separate cluster, which suggested local transmission. Most CAF sequences represented novel, previously unpublished strains.

TABLE 4 Distribution of RSV-A, RSV-B, and untyped cases among RSV-positive cases per year

Year	No. of positive samples typed as RSV-A/Total No. of RSV-positive sample	No. of positive samples typed as RSV-A/Total No. of RSV-positive sample (%)	No. of untyped RSV cases[a]/Total No. of RSV-positive samples (%)
2015	30/52 (57.7)	2/52 (3.8)	20/52 (38.5)
2016	15/64 (23.4)	32/64 (50.0)	17/64 (26.6)
2017	70/115 (60.9)	6/115 (5.2)	39/115 (33.9)
2018	40/81 (49.4)	0/81 (0.0)	41/81 (50.6)
Total	155/312 (49.7)	40/312 (12.8)	117/312 (37.5)

[a]Molecular strain typing was unsuccessful for a certain proportion of RSV-positive samples due to a combination of possible RNA degradation over time and differences in analytical sensitivity between detection and typing PCRs.

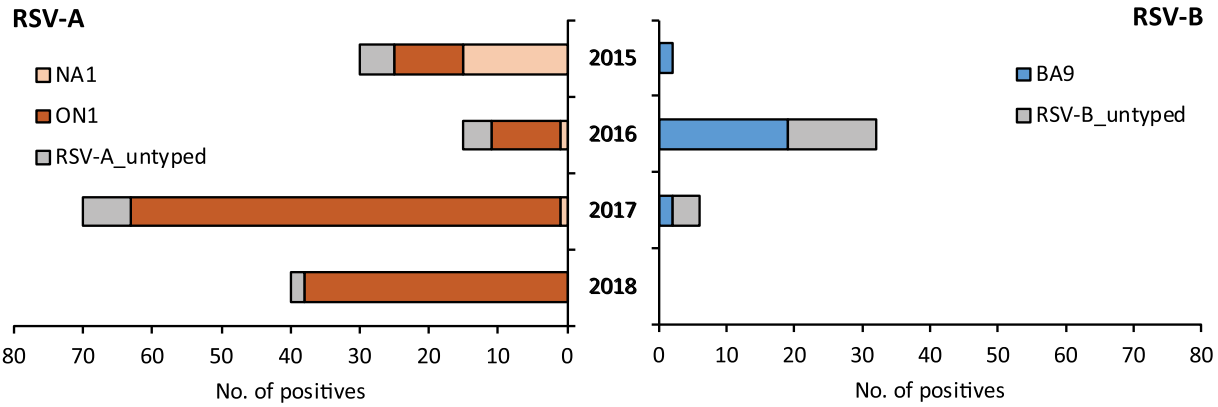

FIGURE 3 Patterns of RSV-A and RSV-B genotype circulation in Central African Republic, 2015 to 2018. Only strains that could be typed as RSV-A or RSV-B are counted (n = 195)

3.7 | Genetic diversity in the second hypervariable region of the G gene

A total of six amino-acid substitutions (D245N, N268S, N281Y, L282P, E292K, and K305I) were identified in the study sequences of the NA1 genotype compared to the prototype strain AB470478 (-Figure S5). A total of 29 amino-acid substitutions were identified in the studied region of the ON1 strains. Eleven mutations (G284S, E286G, L289F, H290Y, E295K, Y297H, P300L, V303A, Y304H, S307P/F) were observed in the duplication region and six (E308K, L310P, S311L, T319N/I, T320A) upstream from the duplication region, six downstream from the duplication region (I243S, T245I, L248I/F, G254R, T259I), and six in the conserved region (E262K, L265H, Y273H, L274P, Y280H, S283F; Figure S6). A total of 22 amino-acid substitutions were observed in the studied BA9 sequences including five upstream from the duplication region (P216L, K218T, L219P, L223P, K233I), two in the conserved region (S247P, T254I), four in the duplication region (T270I, V271A, L272P, D273N), and 11 downstream from the duplication region (I281T, S285F, H287Y, T290N, E292K, S297P, T302A, E305K, P306S, T312N, Q313 stop codon; Figure S7).

4 | DISCUSSION

Our study showed that RSV is an important cause of mild and severe respiratory infections in children under 5 years of age in CAF [2,24,34] and is the first to explore in details the epidemiology of RSV in the country. Young infants are usually more often represented in surveillance studies on respiratory infections, due to the immaturity of their immune system. Compared to the other age groups, children aged 0 to 6 months had an increased risk of being hospitalized regardless of their (RSV) infection status. The detection rate of RSV was high during the first months after birth—13.4% in the 0 to 6 month age group—and decreased with age, to 4.0% in children 4 to 5 years old. Previous reports have shown higher infection rates during the first year of life linked to primary infections.[7,12,35-37] Within the first year of life, a significant difference in hospitalization risk according to the month of birth was shown,[38] linked to infant age and levels of maternal antibodies elicited by more or less recent RSV exposure of the mother.[39,40] RSV infection leads to humoral immune response also in young infants, but antibody titers are likely not high enough to confer full protection. This allows reinfections that will then boost humoral immune response[41,42] and explains why RSV is still detected in older age groups, albeit with lower incidence (4.0%-7.5% in CAF). Our analyses also showed that male patients more frequently developed severe symptoms leading to hospitalization (33.3% vs 29.6% in female) and were more susceptible to RSV infection (9.5% vs 6.4%), as reported previously.[40,43] Hormonal influence on the immune system[44] and/or airway anatomical differences[40] have been proposed as contributing factors to sex differences in susceptibility to respiratory infections. The association of RSV with clinical severity based on a severity scale could only be assessed for a subset of patients with comprehensive clinical records. For those hospitalized children, only young age was associated with higher disease severity, not RSV infection status or gender. The power of the analysis was however likely undermined by the limited number of patients included, the high proportion of intermediate severity scores, and the lack of granularity in the score assessment for parameters such as feeding capacity and chest indrawing for which score 2 could not be attributed due to lack of details in the data recorded. To improve the assessment of RSV disease severity and burden in CAF, healthcare centers participating in the national surveillance should further aim toward more exhaustive clinical data recording for all patients.

Our study showed an overall prevalence of RSV of 8.0% in the period of 2015 to 2018, with significant annual (6.4%-10.6%) and seasonal (12.7% in rainy season vs 3.0% in dry season) fluctuations. While RSV circulation is high during the winter in temperate climates,[12] a high RSV incidence usually coincides with the rainy season in African countries with a tropical climate, such as in Kenya,[45] Cameroon,[46] Senegal,[34] and Ghana.[47] Also in CAF, most cases were reported in November in a study from 2010.[21] RSV seasonality likely depends on climatic factors such as relative humidity, temperature, and UV radiation that influence the infectivity of viral particles and stability of

aerosols[48] or host factors such as overall increased susceptibility to infectious diseases in winter due to lower levels of vitamin D.[49-51] Deviations from overall seasonality patterns, as observed in 2017 in CAF where RSV prevalence in SARI cases was significantly higher with a peak 3 to 5 months earlier than usual, have been documented previously. In Germany, an earlier start of the RSV season as compared to the average tends to be linked to higher disease incidence.[12] In Switzerland and Finland, seasons with lower incidence and later start alternate with a 2 year cycle with outbreaks that start earlier in the season[52,53] have a higher incidence and increased hospitalization rates. Outbreak periodicity is likely influenced by the level of herd immunity developed during previous seasons and/or RSV genetic diversity. In China, RSV-A-dominated seasons started earlier and lasted longer than RSV-B-dominated seasons.[54] Although RSV incidence in 2016 in CAF did not differ from 2015 ($P = .931$) or 2018 ($P = .345$), RSV-B was the predominant subtype in that year, likely affecting herd immunity against RSV-A and thus allowing earlier and wider RSV-A circulation in 2017. Long-term RSV surveillance in CAF is needed to better understand the periodicity of RSV-A and B dominance that differs between countries[54-56] and to refine data on RSV season onset, peak, and duration in the country.

Phylogenetic analyses, based on partial glycoprotein G sequences, revealed an RSV-A genotype replacement in CAF. While NA1 was the dominant genotype in 2015, its detection rates decreased, and it was not found any longer in 2018. RSV temporal genotype replacement is well documented[57-59] and is facilitated by viral evolution and immune selection.[60] Notably, RSV-A ON1 and RSV-B BA genotypes, with a 72 or 60 nt duplication located in the second hypervariable region of the G protein,[4,19,59] have spread worldwide. They have become predominant across different continents,[2,4,19,47,59,61] likely due to a fitness advantage conferred by the duplication.[62] In addition, temporal strain replacement within a genotype also occurred in CAF. Within ON1, one large cluster of 79 identical strains in 2017 to 2018 suggested sustained local transmission. Smaller clusters of identical strains identified during two consecutive seasons (2016-2017 or 2017-2018) or detected in 2016 and 2018 also suggested that these strains were maintained in the country over time. Sporadic cases detected during the dry season, outside the main RSV seasons, may maintain RSV transmission in the population between two outbreaks, without the need for virus reintroduction.[63] However, CAF strains interspersed with RSV strains from abroad, suggesting that new RSV strains are also introduced in the country. Maintaining surveillance in CAF as well as in neighboring countries while increasing the sequencing effort, both concerning the number of strains and the sequence length, will help to characterize the importance of local vs imported strain circulation in the country.

While the NA1 genotype detected in CAF showed limited polymorphism[14,64] potentially contributing to its elimination in CAF,[12] the ON1 and BA9 CAF strains showed a higher degree of polymorphism. Among the 33 substitutions observed in the ON1 CAF strains compared to the prototype strain, L274P substitution has been linked to the RSV evasion from antibodies.[2] Among the 23 mutations in BA9 strains, the genotype-specific substitutions L223P, S247P, I281T, and H287Y[65,66] were also identified in CAF strains. Gain (D245N substitution in three unique NA1 and D273N in three unique BA9 strains) or loss (positions 318-320 in four unique ON1 strains, 296-298 or 310-312 in three BA9 strains) of N-linked glycosylation (-Figures S5-S7) might potentially affect antigenicity.[67,68]

Clinical manifestations of RSV infections vary and can include symptoms of both upper and lower respiratory infections.[69] In our study, RSV was significantly associated with dyspnea, wheezing, chest indrawing, and inability to feed (Table S2) as reported before,[35] while its association with fever or cough could not be assessed due to the use of WHO case definitions developed for influenza surveillance, constituting a limitation of our study.[34] Indeed, a substantial number of RSV-infected patients, especially young infants,[35,70] do not develop fever, while this symptom is part of the ILI and SARI case definitions for influenza surveillance. In the future, allowing the inclusion of children falling within the ARI and extended SARI case definitions should increase sensitivity for RSV case identification.[35,70] Moreover, screening efficiency can be greatly improved by using real-time RT-PCR. While similar detection rates were found in the USA (6.1%)[71] or in Kenya (8%)[45] when using conventional RT-PCR, much higher detection rates were reported in Germany (23%)[12] and in Ghana (23%),[47] when using more sensitive real-time RT-PCR assays.[72] A substantial number of strains could not be further characterized, likely due to the delay between initial diagnostic and further characterization steps, while unstable local long-term storage conditions may have affected nucleic acid quality. Therefore, combining revised RSV surveillance criteria, a more sensitive real-time RT-PCR screening approach as well as optimizing conditions for strain typing will improve the sensitivity of RSV detection and characterization in the country in the future.

5 | CONCLUSION

This first in-depth epidemiological study on RSV in CAF showed concomitant circulation of RSV-A and RSV-B with an alternating predominance and RSV-A genotype replacement from NA1 to ON1. This molecular epidemiological study constitutes a reference for future comparisons of multiyear data to better understand RSV transmission patterns in CAF and to assess the clinical impact of the circulating genotypes. Given preventive palivizumab administration costs and while waiting for a licensed vaccine, awareness and early clinical care remain the best options for preventing severe and deadly RSV infections. Awareness campaigns concerning clinical manifestations of RSV infection, which target mothers of children born within 6 months of RSV peak incidence, should be considered. Comparing epidemiological data with weather data may provide additional insight into the seasonality of RSV infections.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

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The authors have read and approved the final version of the manuscript.

Giscard F. Komoyo had full access to all of the data in the study and takes complete responsibility for the integrity of the data and the accuracy of the data analysis.

TRANSPARENCY STATEMENT

The corresponding author affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

DATA AVAILABILITY STATEMENT

Unique study sequences were deposited in GenBank under the accession numbers HG993989 to HG994028. The information reported in this article is from a database used in a collaborative partnership between the Institut Pasteur of Bangui and the Central African Republic Ministry of Health. The data are not publicly available since they contain information deemed to be confidential for the identity of the included subjects.

ETHICS STATEMENT

The surveillance programme carried out in CAF was approved by the ethical committee from the Ministry of Health, Central African Republic (Decree 0277/MSPP/CAB/DGSPP/DMPM/SMEE of August 5, 2002). Participants were only included in the study after obtaining verbal consent from the child's parents or legal guardians. Data were pseudo-anonymized, in strict compliance with patient privacy rights.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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