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Prevalence and severity of respiratory syncytial virus infections in children in Central African Republic, 2015 to 2018

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August 14, 2020

Abstract

Background: 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). Methods: Taking advantage of the national influenza surveillance network in CAF, 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 to determine annual RSV prevalence, seasonality and disease severity as well as to characterise RSV strains. The presence of RSV viral RNA in nasopharyngeal samples was assessed by RT-PCR, followed by RSV-A and –B typing and Sanger sequencing. Results: RSV incidence 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 2015-2018 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 incidence and peak incidence 3-5 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 were observed. Conclusion: This study represents the first in-depth epidemiological analysis of RSV in CAF and provides first insights into RSV burden, genetic diversity and seasonality in the country.

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 1995-2010, including 20 million 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: the NS1, NS2, N, P, M, SH, G, F, M1-2, M2-2 and L proteins [5]. The surface glycoproteins G and F are the main targets of neutralising 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

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G protein facilitates evasion of the immune response, allowing re-infections 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, there are 15 genotypes in the RSV-A sub-group (GA1-GA7, SAA1-SAA2, CB-A, NA1-NA4, and ON1) and 37 genotypes in the B sub-group (GB1-GB13, SAB1-SAB4, URU1-URU2, CB1, CBB, JAB1, THB and BA1-BA14)[14-17]. In the RSV-A sub-group, the ON1 genotype was first described in 2010 in Canada and is the most frequent RSV-A genotype to date. It contains a 72 nucleotide (nt) duplication in the gene sequence corresponding to the C-terminal region of the G protein, the largest known duplication [2,4]. In RSV-B, the 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), a preliminary study reported RSV in 3.0% (10/329) of children aged 0-15 years enrolled from January to December 2010 [20]. Here, we analysed epidemiological and clinical data and characterised RSV strains from children under 5 years of age, hospitalized or not, recruited in 5 sentinel sites and provide first insights into RSV prevalence, seasonality and disease severity in CAF.

Methods

Sample collection

This study was carried out as part of the national influenza surveillance programme in CAF. Samples were collected from children under 5 years of age who came to the Bangui Paediatric Complex for consultation for a severe acute respiratory illness (SARI) syndrome or to four other sentinel sites for an influenza-like illness (ILI) syndrome. From January 2015 to December 2018, a total of 3903 nasopharyngeal swabs were collected from patients meeting 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 [?]38degC and cough with onset within the last 10 days requiring hospitalization) (Fitzner et al., 2018). The swab was placed in a labelled tube with 3 mL of Universal Transport Medium (UTM; Copan, Italy) and stored at 4degC or sent immediately in a cold box to the National Reference Centre (NRC) 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 -80degC. Social, demographic, clinical and epidemiological data were recorded for each patient using a standardised questionnaire.

Nucleic acid extraction and RSV detection by RT-PCR

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 protocol. RSV detection was performed by a conventional multiplex one-step RT-PCR detecting human metapneumovirus, influenza A and influenza B virus as described before [21]. Cycling conditions were as follows: 50°C for 30 min, 94°C for 15 min followed by 40 cycles of 94°C for 30 sec, 55°C for 30 sec, 72°C for 1 min, and a final extension at 72°C for 10 min.

RSV typing and sequencing

RSV positive samples were then typed as RSV-A or -B by targeting fusion protein F by a semi-nested PCR as described before [22]. cDNA synthesis was carried out using the SuperScript III First-Strand enzyme (Invitrogen, USA) and the anti-sense primer F164 (5'-GTTATGACACTGGTATACCAACC-3') [22].

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

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were then performed on each sample with the AG655 (5'-GATCYCAAACCTCAAACCAC-3') or BG517 (5'-TTYGTTCCCTGTAGTATATGTG-3') sense primers and F164 antisense primer [22].

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 pb 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.

Data availability

Unique study sequences were deposited in GenBank under the accession numbers xxx to xxx.

Phylogenetic analysis

Consensus sequences were generated using SeqScape software (version 2.5) and analysed with BLAST (*http://www.ncbi.nlm.nih.gov.BLAST/*) for similarity searches. All sequences were aligned using ClustalW implemented in BioEdit v7.2.5 [23]. 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[24], and used to calculate the phylogenetic trees with the Maximum Likelihood method with 1000 bootstrap iterations as implemented in MEGA v6.06.

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 visualise the amino-acid variability in the second hypervariable part of the G protein.

Glycosylation sites

Potential glycosylation sites in the second hypervariable part of the G protein were identified using NetNGlyc 1.0 server (http://www.cbs.dtu.dk/services/NetNGlyc) and NetOGlyc 4.0 server (http://www.cbs.dtu.dk/services/NetOGlyc). Oxygen (O)-linked glycosylation sites occur on serine (S) and threonine (T) and nitrogen (N)-linked glycosylation sites occur on N-X-S/T, where X is any amino acid other than proline (P).

Statistical analyses

Statistical analyses were performed in SigmaPlot v12.5, using Mann-Whitney rank sum tests for continuous variables, χ2-test for categorical variables or z-test for low proportions. Odds ratios were calculated with χ2-test with the Yates correction.

Ethical considerations

The surveillance programme carried out in CAF was approved by an ethics committee composed of experts from the Ministry of Health (Decree 0277/MSPP/CAB/DGSPP/DMPM/SMEE of 5 August 2002). Participants were only included in the study after obtaining verbal consent from the child's parents or legal guardians. Data were pseudo-anonymised, in strict compliance with patient privacy rights. The results were sent to the child's regular medical practitioner in a sealed envelope.

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Results

Demographic and clinical characteristics of patients

From January 2015 to December 2018, 3903 patients with a respiratory syndrome corresponding to the SARI (31.5%) or ILI (68.5%) case definitions were included in the study. The demographic and clinical characteristics for enrolled patients are given 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). ILI cases were mainly recruited at 4 out of 5 sentinel centers. SARI cases were mainly recruited (56.1%) at the Bangui Paediatric Complex, a hospital that is specialized in pediatric care, while between 3.8 to 16.5% of SARI cases were recruited in the other 4 centers. 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 ($p<0.001$), while females had a lower risk of presenting with SARI compared to male (OR=0.84, p=0.014; Table S1).

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). Among these 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. Of the 155 RSV-A samples, 52/155 (33.5%) were from ILI cases and 103/155 (66.4%) were from SARI cases, with an overrepresentation of SARI cases in 2017. Of the 40 RSV-B, 13/40 (32.5%) were ILI patients compared with 27/40 (67.5%) SARI cases.

Among the RSV-positive cases, increased RSV detection in males (9.5%) compared to females (6.4%) was observed (OR= 1.53, $p<0.001$; Table 3). Children aged 0-6 months (13.4%) had an increased risk to be RSV positive compared to all other age groups (4.0-7.5%; $p<0.001$; Table 3).

Disease severity and RSV related deaths

RSV positive cases were more frequently detected among hospitalized patients (13.3% vs 5.5%, OR=2.62, $p<0.001$; Table 3). RSV was associated with dyspnea ($p<0.001$), wheezing ($p<0.001$), chest indrawing ($p<0.001$) and inability to feed (p=0.002) but not with rhinorrhoea (p=0.993), diarrhoea (p=0.983), vomiting (p=0.107), lethargy (p=0.816) or convulsion (p=0.752; Table 3). In hospitalized children, RSV was significantly more frequently detected in children with a diagnosis of bronchiolitis or bronchopneumonia compared to pneumonia (Table 3), RSV had a significant influence on oxygen saturation levels (RSV neg, n=294, median SaO_2=96% *vs* RSV pos, n=64, median SaO_2=92%; p=0.049; SaO_2 levels not determined, n=874) and was associated with increased duration of hospitalisation ($p<0.001$).

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=0.162). For these 8 patients all younger than 24 months, delay between symptom onset and hospitalisation ranged from 0-7 days while the delay until hospitalisation and death ranged from 0-9 days. Co-morbidities were reported for 4 patients and included malnutrition, malaria and congenital heart disease. When measured (5/8), oxygen saturation levels were <95%. Bacterial co-infections with *Staphylococcus aureus* , *Haemophilus influenzae* , *Moraxella catarrhalis* ,*Streptococcus pneumoniae* and/or co-infections with influenza A virus were detected in 5 patients (Table S2).

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 in 2017 was significantly higher compared to 2015 (p=0.002), 2016 (p=0.002) and 2018 (p=0.031).

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During the study period, RSV detections started in March-April and lasted until December (Fig. 1 and Fig. 2). Sporadic detections were recorded between March-April to 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 (Fig. 2). RSV was also significantly more frequently detected in the rainy season compared to the dry season (255/1756, 12.7% vs 57/1892, 3.0%; p<0.001).

Among the RSV strains that could be typed, 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 2015-2017 and was predominant in 2016, representing more than half of the RSV-positive cases (32/47, 68.1%; Fig. 3; Table 2).

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 (Fig. 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 (Fig. 3).

Genetic distances between strains of the same genotype ranged from 0-1.4% for RSV-A NA1, 0-6.3% for RSV-A ON1 and 0-6.4% for RSV-B BA9. Five unique NA1 sequences (5/17, 29.4%) were observed and the same strain was found in 2015 to 2017 (Fig. S1). Twenty-six unique nucleotide sequences clustered within ON1 genotype (26/120, 21.7%). The same strains were found over one or two years on only four occasions, while a large cluster of identical strains (n=79) was found in 2017-2018 which might suggest local transmission. Ten unique BA9 sequences were identified (10/23, 43.5%; Fig. S2). Four unique strains were interspersed within the BA9 genotype, while the other 6 unique strains (corresponding to 18 sequences in total) formed a separate cluster, which suggested local transmission. Most CAF sequences represented novel, previously unpublished strains.

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 (Fig. S3). 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; Fig. S4). 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; Fig. S5).

Profile of potentials N and O-glycosylated sites

Analysis of potentials N- and O-glycosylated sites showed distinct patterns of glycosylation sites among CAF isolates.

Among RSV type A isolates a new N-glycosylated site (NTT code) appeared at amino acid position 245 of isolates of NA1 genotypes. The other N-glycosylated sites (position 259 and 302 of NA1 genotype, fig. S3; position 247 and 318 of ON1 genotype, fig. S4) were identical to the prototype strains.

In RSV type B isolates, a new N-glycosylated site (NTT code) appeared in the duplicate area at amino acid position 273 (Fig. S5).

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The O-glycosylation profile was similar when 22 amino acid sequences for the ON1 genotype G protein and 3 amino acids for the CAF NA1 genotype G protein were analyzed. The O-glycosylation profile predicted (402 sites for ON1 and 102 sites for NA1) serine and threonine and would be O-glycosylated with a score of 0.5-0.7.

Analysis of O-glycosylated amino acid sites of 7 strains of RSV-B predicted up to 77 potential O-glycosylated sites of serine and threonine residues with a score of 0.5-0.7.

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[2,22,25] 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 0-6 months had an increased risk of being hospitalized regardless of their (RSV) infection status, and the detection rate of RSV was high during the first months after birth — 13.4% in the 0–6 month age group — and decreased with age, to 4.0% in children 4-5 years old. Previous reports have shown higher infection rates during the first year of life linked to primary infections[7,12,26-28]. Within the first year of life, a significant difference in hospitalization risk according to the month of birth was shown [29], linked to infant age and levels of maternal antibodies elicited by more or less recent RSV exposure of the mother [30,31]. 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 [32,33] and explaining 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 developed more frequently more severe symptoms leading to hospitalisation (33.3% vs 29.6% in female) and were more susceptible to RSV infection (9.5% vs 6.4%), as already reported previously[31,34]. Hormonal influence on the immune system[35] and/or airway anatomical differences[31] have been proposed to contribute to sex differences in susceptibility to respiratory infections.

Our study showed an overall prevalence of RSV of 8.0% in the period of 2015-2018, 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 [36], Cameroon [37], Senegal[25] and Ghana [38]. Also in CAF most cases were reported in November in a study from 2010[20]. 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[39] or host factors such as overall increased susceptibility to infectious diseases in winter due to lower levels of vitamin D [40-42]. Deviations from overall seasonality patterns, as observed in 2017 in CAF where RSV incidence was significantly higher with a peak 3-5 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 [43,44], have a higher incidence and increased hospitalisation rates[43]. 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 [45]. Although RSV incidence in 2016 in CAF did not differ from 2015 (p=0.931) or 2018 (p=0.345), 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 [45-47], 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 [48-50] and is facilitated

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by viral evolution and immune selection [51]. 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 [4,19,50], have spread worldwide. They have become predominant across different continents [2,4,19,38,50,52], likely due to a fitness advantage conferred by the duplication [53]. In addition, temporal strain replacement within a genotype also occurred in CAF. Within ON1, one large cluster of 79 identical strains in 2017-2018 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 re-introduction [54]. 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 neighbouring countries while increasing the sequencing effort, both concerning the number of strains and the sequence length, will help to characterize the importance of local versus imported strain circulation in the country.

While the NA1 genotype detected in CAF showed limited polymorphism[14,55] 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 [56,57] were also identified in CAF strains. Gain (D245N substitution in 3 unique NA1 and D273N in 3 unique BA9 strains) or loss (positions 318-320 in 4 unique ON1 strains, 296-298 or 310-312 in 3 BA9 strains) of N-linked glycosylation might potentially affect antigenicity[58,59].

Clinical manifestations of RSV infections vary and can include symptoms of both upper and lower respiratory infections [60]. In our study, RSV was significantly associated with dyspnea, wheezing, chest indrawing and inability to feed as reported before[26], 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[25]. Indeed, a substantial number of RSV infected patients, especially young infants [26,61], do not develop fever, while this symptom is part of the ILI and SARI case definitions. Using ARI and extended SARI definitions should increase sensitivity for RSV case identification [26,61]. Moreover, screening efficiency can be greatly improved by using real-time RT-PCR. While similar detection rates were found in the USA (6.1%, [62]) or in Kenya (8%; [36]) when using conventional RT-PCR, much higher detection rates were reported in Germany (23%; [12]) and in Ghana (23%;[38]), when using more sensitive real-time RT-PCR assays[63]. Therefore, combining revised RSV surveillance criteria and a more sensitive real-time RT-PCR screening approach will improve the sensitivity of RSV detection in the country in the future.

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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Acknowledgments

The authors wish to thank L. Ngombi for taking the samples from the CPB, B. Selekon, A. Sausy and R. Sinner for technical help.

This study was supported by the Department of Health and Human Service (DHHS) of USA, by the Luxembourg Ministry of Foreign and European Affairs and Luxembourg Institute of Health [grants MAE-IV and MAE-V]. G.F. Komoyo was also supported by a PhD training fellowship funded by the Luxembourg Ministry of Foreign and European Affairs.

Conflict of interest

The authors declare that they have no conflict of interests.

References

1. Chanock RM. Association of a new type of cytopathogenic myxovirus with infantile croup. *J Exp Med.* Oct 1 1956;104(4):555-576.

2. Ahmed A, Haider SH, Parveen S, et al. Co-Circulation of 72bp Duplication Group A and 60bp Duplication Group B Respiratory Syncytial Virus (RSV) Strains in Riyadh, Saudi Arabia during 2014. *PLoS One.* 2016;11(11):e0166145.

3. Baek YH, Choi EH, Song MS, et al. Prevalence and genetic characterization of respiratory syncytial virus (RSV) in hospitalized children in Korea. *Arch Virol.* Jun 2012;157(6):1039-1050.

4. Eshaghi A, Duvvuri VR, Lai R, et al. Genetic variability of human respiratory syncytial virus A strains circulating in Ontario: a novel genotype with a 72 nucleotide G gene duplication. *PLoS One.*2012;7(3):e32807.

5. Pangesti KNA, Abd El Ghany M, Walsh MG, Kesson AM, Hill-Cawthorne GA. Molecular epidemiology of respiratory syncytial virus. *Rev Med Virol.* Mar 2018;28(2).

6. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory infections in 195 countries, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Infect Dis.* Nov 2018;18(11):1191-1210.

7. Nair H, Nokes DJ, Gessner BD, et al. Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. *Lancet.* May 1 2010;375(9725):1545-1555.

8. Shi T, McAllister DA, O'Brien KL, et al. Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: a systematic review and modelling study. *Lancet.* Sep 2 2017;390(10098):946-958.

9. Vieira SE, Thomazelli LM, de Paulis M, et al. Infections Caused by HRSV A ON1 Are Predominant among Hospitalized Infants with Bronchiolitis in Sao Paulo City. *Biomed Res Int.* 2017:3459785.

10. Tripp RA, Jones LP, Haynes LM, Zheng H, Murphy PM, Anderson LJ. CX3C chemokine mimicry by respiratory syncytial virus G glycoprotein. *Nat Immunol.* Aug 2001;2(8):732-738.

11. Parveen S, Broor S, Kapoor SK, Fowler K, Sullender WM. Genetic diversity among respiratory syncytial viruses that have caused repeated infections in children from rural India. *J Med Virol.*May 2006;78(5):659-665.

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12. Reiche J, Schweiger B. Genetic variability of group A human respiratory syncytial virus strains circulating in Germany from 1998 to 2007. *J Clin Microbiol.* Jun 2009;47(6):1800-1810.

13. Venter M, Madhi SA, Tiemessen CT, Schoub BD. Genetic diversity and molecular epidemiology of respiratory syncytial virus over four consecutive seasons in South Africa: identification of new subgroup A and B genotypes. *J Gen Virol.* Sep 2001;82(Pt 9):2117-2124.

14. Fan R, Fan C, Zhang J, et al. Respiratory syncytial virus subtype ON1/NA1/BA9 predominates in hospitalized children with lower respiratory tract infections. *J Med Virol.* Feb 2017;89(2):213-221.

15. Ahmed A, Parveen S, Al-Hassinah SM, Al-Amery SF. An overview of respiratory syncytial virus infections in Saudi Arabia.*J Infect Dev Ctries.* Nov 30 2018;12(11):929-936.

16. Zhang ZY, Du LN, Chen X, et al. Genetic variability of respiratory syncytial viruses (RSV) prevalent in Southwestern China from 2006 to 2009: emergence of subgroup B and A RSV as dominant strains.*J Clin Microbiol.* Apr 2010;48(4):1201-1207.

17. Ren L, Xiao Q, Zhou L, Xia Q, Liu E. Molecular characterization of human respiratory syncytial virus subtype B: a novel genotype of subtype B circulating in China. *J Med Virol.* Jan 2015;87(1):1-9.

18. Haider MSH, Khan WH, Deeba F, et al. BA9 lineage of respiratory syncytial virus from across the globe and its evolutionary dynamics. *PLoS One.* 2018;13(4):e0193525.

19. Trento A, Galiano M, Videla C, et al. Major changes in the G protein of human respiratory syncytial virus isolates introduced by a duplication of 60 nucleotides. *J Gen Virol.* Nov 2003;84(Pt 11):3115-3120.

20. Nakoune E, Tricou V, Manirakiza A, et al. First introduction of pandemic influenza A/H1N1 and detection of respiratory viruses in pediatric patients in Central African Republic. *Virol J.* Feb 8 2013;10:49.

21. Buecher C, Mardy S, Wang W, et al. Use of a multiplex PCR/RT-PCR approach to assess the viral causes of influenza-like illnesses in Cambodia during three consecutive dry seasons. *J Med Virol.* Oct 2010;82(10):1762-1772.

22. Parveen S, Sullender WM, Fowler K, Lefkowitz EJ, Kapoor SK, Broor S. Genetic variability in the G protein gene of group A and B respiratory syncytial viruses from India. *J Clin Microbiol.* Sep 2006;44(9):3055-3064.

23. Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids. Symp. Ser.* 1999;41:95-98.

24. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol Biol Evol.* Dec 2013;30(12):2725-2729.

25. Fall A, Dia N, Cisse el HA, et al. Epidemiology and Molecular Characterization of Human Respiratory Syncytial Virus in Senegal after Four Consecutive Years of Surveillance, 2012-2015.*PLoS One.* 2016;11(6):e0157163.

26. Hirve S, Crawford N, Palekar R, Zhang W, Group WRs. Clinical characteristics, predictors, and performance of case definition-Interim results from the WHO global respiratory syncytial virus surveillance pilot. *Influenza Other Respir Viruses.* Oct 31 2019.

27. Kwofie TB, Anane YA, Nkrumah B, Annan A, Nguah SB, Owusu M. Respiratory viruses in children hospitalized for acute lower respiratory tract infection in Ghana. *Virol J.* Apr 10 2012;9:78.

28. Mohamed GA, Ahmed JA, Marano N, et al. Etiology and Incidence of Viral Acute Respiratory Infections Among Refugees Aged 5 Years and Older in Hagadera Camp, Dadaab, Kenya. *Am J Trop Med Hyg.* Dec 2015;93(6):1371-1376.

29. Viguria N, Martinez-Baz I, Moreno-Galarraga L, Sierrasesumaga L, Salcedo B, Castilla J. Respiratory syncytial virus hospitalization in children in northern Spain. *PLoS One.*2018;13(11):e0206474.

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30. Birkhaug IM, Inchley CS, Aamodt G, Anestad G, Nystad W, Nakstad B. Infectious burden of respiratory syncytial virus in relation to time of birth modifies the risk of lower respiratory tract infection in infancy: the Norwegian Mother and Child Cohort. *Pediatr Infect Dis J.* Jun 2013;32(6):e235-241.

31. Sommer C, Resch B, Simoes EA. Risk factors for severe respiratory syncytial virus lower respiratory tract infection.*Open Microbiol J.* 2011;5:144-154.

32. Botosso VF, Zanotto PM, Ueda M, et al. Positive selection results in frequent reversible amino acid replacements in the G protein gene of human respiratory syncytial virus. *PLoS Pathog.* Jan 2009;5(1):e1000254.

33. Gonzalez PA, Bueno SM, Carreno LJ, Riedel CA, Kalergis AM. Respiratory syncytial virus infection and immunity. *Rev Med Virol.* Jul 2012;22(4):230-244.

34. Gaymard A, Bouscambert-Duchamp M, Pichon M, et al. Genetic characterization of respiratory syncytial virus highlights a new BA genotype and emergence of the ON1 genotype in Lyon, France, between 2010 and 2014. *J Clin Virol.* May 2018;102:12-18.

35. Falagas ME, Mourtzoukou EG, Vardakas KZ. Sex differences in the incidence and severity of respiratory tract infections. *Respir Med.* Sep 2007;101(9):1845-1863.

36. Okiro EA, Ngama M, Bett A, Nokes DJ. The incidence and clinical burden of respiratory syncytial virus disease identified through hospital outpatient presentations in Kenyan children. *PLoS One.* 2012;7(12):e52520.

37. Kenmoe S, Vernet MA, Miszczak F, et al. Genetic diversity of human respiratory syncytial virus isolated among children with acute respiratory infections in Southern Cameroon during three consecutive epidemic seasons, 2011-2013. *Trop Med Health.* 2018;46:7.

38. Obodai E, Odoom JK, Adiku T, et al. The significance of human respiratory syncytial virus (HRSV) in children from Ghana with acute lower respiratory tract infection: A molecular epidemiological analysis, 2006 and 2013-2014. *PLoS One.* 2018;13(9):e0203788.

39. Yusuf S, Piedimonte G, Auais A, et al. The relationship of meteorological conditions to the epidemic activity of respiratory syncytial virus. *Epidemiol Infect.* Oct 2007;135(7):1077-1090.

40. Cannell JJ, Vieth R, Umhau JC, et al. Epidemic influenza and vitamin D. *Epidemiol Infect.* Dec 2006;134(6):1129-1140.

41. Klotman ME, Chang TL. Defensins in innate antiviral immunity. *Nat Rev Immunol.* Jun 2006;6(6):447-456.

42. Wang TT, Nestel FP, Bourdeau V, et al. Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. *J Immunol.* Sep 1 2004;173(5):2909-2912.

43. Duppenthaler A, Gorgievski-Hrisoho M, Frey U, Aebi C. Two-year periodicity of respiratory syncytial virus epidemics in Switzerland. *Infection.* Mar 2003;31(2):75-80.

44. Gunell M, Antikainen P, Porjo N, et al. Comprehensive real-time epidemiological data from respiratory infections in Finland between 2010 and 2014 obtained from an automated and multianalyte mariPOC(R) respiratory pathogen test. *Eur J Clin Microbiol Infect Dis.* Mar 2016;35(3):405-413.

45. Yu J, Liu C, Xiao Y, et al. Respiratory Syncytial Virus Seasonality, Beijing, China, 2007-2015. *Emerg Infect Dis.* Jun 2019;25(6):1127-1135.

46. de-Paris F, Beck C, de Souza Nunes L, et al. Evaluation of respiratory syncytial virus group A and B genotypes among nosocomial and community-acquired pediatric infections in Southern Brazil. *Virol J.* Feb 24 2014;11:36.

47. Wansaula Z, Olsen SJ, Casal MG, et al. Surveillance for severe acute respiratory infections in Southern Arizona, 2010-2014.*Influenza Other Respir Viruses.* May 2016;10(3):161-169.

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48. Munoz-Escalante JC, Comas-Garcia A, Bernal-Silva S, Robles-Espinoza CD, Gomez-Leal G, Noyola DE. Respiratory syncytial virus A genotype classification based on systematic intergenotypic and intragenotypic sequence analysis. *Sci Rep.* Dec 27 2019;9(1):20097.

49. Pretorius MA, van Niekerk S, Tempia S, et al. Replacement and positive evolution of subtype A and B respiratory syncytial virus G-protein genotypes from 1997-2012 in South Africa. *J Infect Dis.*Dec 15 2013;208 Suppl 3:S227-237.

50. Trento A, Casas I, Calderon A, et al. Ten years of global evolution of the human respiratory syncytial virus BA genotype with a 60-nucleotide duplication in the G protein gene. *J Virol.* Aug 2010;84(15):7500-7512.

51. Choi EH, Lee HJ. Genetic diversity and molecular epidemiology of the G protein of subgroups A and B of respiratory syncytial viruses isolated over 9 consecutive epidemics in Korea.*J Infect Dis.* May 2000;181(5):1547-1556.

52. Cui G, Zhu R, Qian Y, et al. Genetic variation in attachment glycoprotein genes of human respiratory syncytial virus subgroups a and B in children in recent five consecutive years.*PLoS One.* 2013;8(9):e75020.

53. Hotard AL, Laikhter E, Brooks K, Hartert TV, Moore ML. Functional Analysis of the 60-Nucleotide Duplication in the Respiratory Syncytial Virus Buenos Aires Strain Attachment Glycoprotein. *J Virol.* Aug 2015;89(16):8258-8266.

54. Zlateva KT, Lemey P, Vandamme AM, Van Ranst M. Molecular evolution and circulation patterns of human respiratory syncytial virus subgroup a: positively selected sites in the attachment g glycoprotein.*J Virol.* May 2004;78(9):4675-4683.

55. Haider MSH, Deeba F, Khan WH, et al. Global distribution of NA1 genotype of respiratory syncytial virus and its evolutionary dynamics assessed from the past 11years. *Infect Genet Evol.* Jun 2018;60:140-150.

56. Dapat IC, Shobugawa Y, Sano Y, et al. New genotypes within respiratory syncytial virus group B genotype BA in Niigata, Japan.*J Clin Microbiol.* Sep 2010;48(9):3423-3427.

57. Tabatabai J, Prifert C, Pfeil J, Grulich-Henn J, Schnitzler P. Novel respiratory syncytial virus (RSV) genotype ON1 predominates in Germany during winter season 2012-13. *PLoS One.*2014;9(10):e109191.

58. Garcia-Beato R, Martinez I, Franci C, Real FX, Garcia-Barreno B, Melero JA. Host cell effect upon glycosylation and antigenicity of human respiratory syncytial virus G glycoprotein.*Virology.* Jul 15 1996;221(2):301-309.

59. Lambert DM. Role of oligosaccharides in the structure and function of respiratory syncytial virus glycoproteins. *Virology.*Jun 1988;164(2):458-466.

60. Welliver RC. Review of epidemiology and clinical risk factors for severe respiratory syncytial virus (RSV) infection. *J Pediatr.* Nov 2003;143(5 Suppl):S112-117.

61. Rha B, Dahl RM, Moyes J, et al. Performance of Surveillance Case Definitions in Detecting Respiratory Syncytial Virus Infection Among Young Children Hospitalized With Severe Respiratory Illness-South Africa, 2009-2014. *J Pediatric Infect Dis Soc.* Sep 25 2019;8(4):325-333.

62. Fowlkes A, Giorgi A, Erdman D, et al. Viruses associated with acute respiratory infections and influenza-like illness among outpatients from the Influenza Incidence Surveillance Project, 2010-2011. *J Infect Dis.* Jun 1 2014;209(11):1715-1725.

63. Templeton KE, Scheltinga SA, van den Eeden WC, Graffelman AW, van den Broek PJ, Claas EC. Improved diagnosis of the etiology of community-acquired pneumonia with real-time polymerase chain reaction.*Clin Infect Dis.* Aug 1 2005;41(3):345-351.

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Figure legends

Fig. 1 : Distribution of RSV cases by month over the four years of the study

Fig. 2 : Overlap of RSV seasons over the four years (2015-2018) of the study and overall dry/rainy season distinction

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

Tables

Table 1: Demographic characteristics of patients included in the study by year

Demographic characteristics	**2015**	**2016**	**2017**	**2018**	**Total**
Total number	**(N =813)**	**(N = 968)**	**(N =1084)**	**(N =1038)**	**(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 (%)					
0-6 months	253 (31.1)	261 (27.0)	318 (29.3)	226 (21.8)	1058 (27.1)
7-12 months	130 (16.0)	150 (15.5)	177 (16.3)	206 (19.8)	663 (17.0)
13-24 months	157 (19.3)	216 (22.3)	239 (22.0)	218 (21.0)	830 (21.3)
25-36 months	128 (15.7)	154 (15.9)	170 (15.7)	194 (18.7)	646 (16.6)
37-48 months	81 (10.0)	100 (10.3)	91 (8.4)	107 (10.3)	379 (9.7)
49-60 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)
Bangui 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)

Table 2: Distribution of the RSV sub-groups (RSV-A and RSV-B) according to study year and syndrome type

Year	**Total RSV detections**	**RSV-A**			**RSV-B**			**Not typed**
		ILI	**SARI**	**Total**	**ILI**	**SARI**	**Total**	**Total**
	n/N (%)	n	n	n/N (%)	n	n	n/N (%)	n/N (%)
2015	52/813 (6.4)	16	14	30/52 (57.7)	2	0	2/52 (3.8)	20/52 (38.5)
2016	64/968 (6.6)	6	9	15/64 (23.4)	11	21	32/64 (50.0)	17/64 (26.6)
2017	115/1084 (10.6)	6	64	70/115 (60.9)	0	6	6/115 (5.2)	39/115 (33.9)
2018	81/1038 (7.8)	24	16	40/81 (49.4)	0	0	0/81 (0.0)	41/81 (50.6)
Total	312/3903 (8.0)	52	103	155/312 (49.7)	13	27	40/312 (12.8)	117/312 (37.5)

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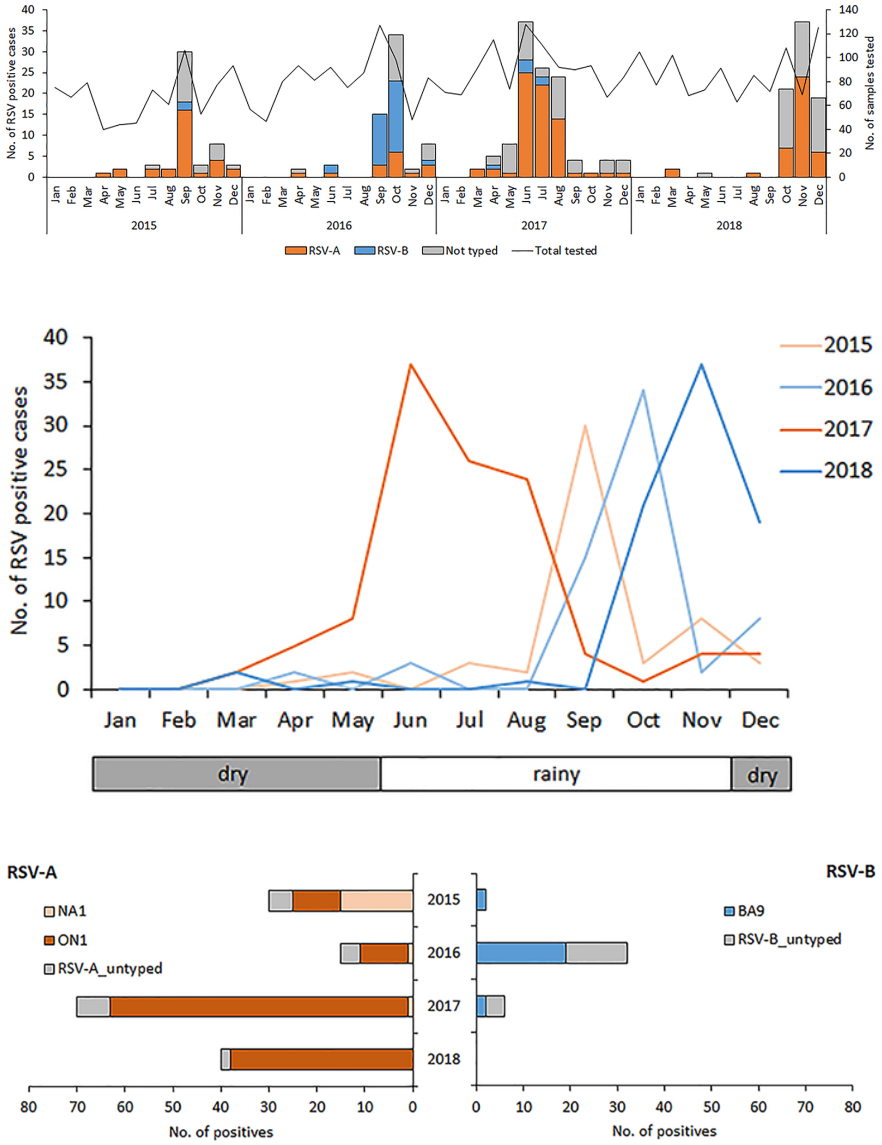
Table 3 : RSV prevalence in children and evaluation of characteristics associated with increased odds of RSV detection

Variable		n/N (%)	OR (95% CI)	p value
Sex				
F		120/1877 (6.4)	ref	
M		192/2026 (9.5)	1.53 (1.21 - 1.94)	p<0.001
Age group				
0-6 months		142/1058 (13.4)	ref	
7-12 months		50/663 (7.5)	0.53 (0.38 - 0.74)	p<0.001
13-24 months		50/830 (6.0)	0.41 (0.30 - 0.58)	p<0.001
25-36 months		36/646 (5.6)	0.38 (0.26 - 0.56)	p<0.001
37-48 months		21/379 (5.5)	0.38 (0.24 - 0.61)	p<0.001
49-60 months		13/327 (4.0)	0.27 (0.15 - 0.48)	p<0.001
Clinical case				
ILI		148/2672 (5.5)	ref	
SARI		164/1231 (13.3)	2.62 (2.08 - 3.31)	p<0.001
Clinical symptoms				
rhinorrhoea				
	no	27/332 (8.1)	ref	
	yes	285/3571 (8.0)	0.98 (0.65 - 1.48)	p=0.993
dyspnea				
	no	183/2987 (6.1)	ref	
	yes	129/916 (14.1)	2.51 (1.98 - 3.19)	p<0.001
wheezing				
	no	224/3444 (6.5)	ref	
	yes	88/459 (19.2)	3.41 (2.61 - 4.47)	p<0.001
chest indrawing				
	no	262/3582 (7.3)	ref	
	yes	50/321 (15.6)	2.34 (1.69 - 3.24)	p<0.001
diarrhoea				
	no	238/2973 (8.0)	ref	
	yes	74/930 (8.0)	0.99 (0.76 - 1.30)	p=0.983
vomiting				
	no	253/3295 (7.7)	ref	
	yes	59/608 (9.7)	1.29 (0.96 - 1.74)	p=0.107
lethargy				
	no	310/3867 (8.0)	ref	
	yes	2/36 (5.6)	0.68 (0.16 - 2.82)	p=0.816
inability to feed				
	no	258/3447 (7.5)	ref	
	yes	54/456 (11.8)	1.66 (1.22 - 2.27)	p=0.002
convulsion				
	no	294/3699 (7.9)	ref	
	yes	18/204 (8.8)	1.12 (0.68 - 1.85)	p=0.752
Diagnosis upon admission*	**Diagnosis upon admission***			
Pneumonia		45/332 (13.6)	ref	
Bronchopneumonia	Bronchopneumonia	14/46 (30.4)	2.79 (1.32 - 5.63)	p=0.006
Bronchiolitis		63/311 (20.3)	1.62 (1.07 - 2.46)	p=0.030
Not determined or others	Not determined or others	42/542 (7.7)	-	-

* for hospitalized children only



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