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Original Article

Outbreak of respiratory syncytial virus subtype ON1 among children during COVID-19 pandemic in Southern Taiwan

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Abstract *Background:* The regional respiratory syncytial virus (RSV) outbreak in southern Taiwan in late 2020 followed the surge of RSV cases in the national surveillance data and displayed distinct clinical features. This study investigated RSV epidemiology in the most recent five years and compared the clinical manifestations of this outbreak with non-outbreak period.
Methods: Medical records of RSV-infected children at the National Cheng Kung University Hospital from January 2016 to December 2020 were retrospectively retrieved from hospital-based electronic medical database. Cases of RSV infection were identified by RSV antigen positive and/or RSV isolated from respiratory specimens. The demographic, clinical presentations, and laboratory data were recorded. The RSV isolates in 2020 was sequenced for phylogenetic analysis.
Results: Overall, 442 RSV-infected cases were retrieved and 42.1% (186 cases) clustered in late 2020. The 2020 outbreak started in September, peaked in November, and lasted for 3 months. 2020 RSV-infected children were older (2.3 ± 2.2 years vs. 1.0 ± 1.0 years), more likely to be

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diagnosed with bronchopneumonia (57.5% vs. 31.6%), but also had a lower hospitalization rate, shorter hospital stay, less oxygen use, and less respiratory distress than those in 2016—2019 (all p value < 0.05). The RSV isolates in 2020 belonged to RSV-A subtype ON1 but were phylogenetically distinct from the ON1 strains prevalent in Taiwan previously.
Conclusion: The 2020 RSV outbreak was led by the novel RSV-A subtype ON1 variant with clinical manifestations distinct from previous years. Continuous surveillance of new emerging variants of respiratory viruses in the post-pandemic era is warranted.

Introduction

Respiratory syncytial virus (RSV) is a leading cause of respiratory tract infection (RTI) in infants and young children. Nearly every child gets RSV infection at least once by the age of two.[1] In the United States, RSV is responsible for 57,000 hospitalizations and 500,000 emergency department visits among children <5 years old annually.[2] RSV is an enveloped, negative-sense, single-stranded RNA virus in the *Pneumoviridae* family with a genome encoding for 11 proteins. Out of those, the external glycoproteins G and F are involved in attachment and entry into the host cells.[3] Glycoprotein G, or G protein, is composed of a central conserved domain and two hypervariable regions containing many N- and O-glycosylation sites that contribute to antigenicity. G proteins are classified as A and B, based on the genetic variability of the second hypervariable region, and at least 13 RSV-A genotypes and 20 RSV-B genotypes have been identified on the basis of G protein gene sequences.[4]

In temperate climates, RSV infection rates typically peak during the cold season, whereas in tropical climates RSV infection rates usually peak during the rainy season.[5,6] A study conducted between 2001 and 2005 found RSV infection to occur biennially, with peaks in spring and fall, in northern Taiwan.[7] These results were further supported by another study conducted between 2004 and 2007, using Taiwan's National Health Insurance database.[8] However, there is limited epidemiological data on the seasonality of RSV infection in southern Taiwan.

Public health measures taken in response to the coronavirus disease 2019 (COVID-19) pandemic, including universal mask wearing and social distancing targeting, also decreased the transmission of other respiratory viruses. Yeoh et al. reported decreased influenza and RSV detections in Western Australian (WA) children after public health measures were introduced during the COVID-19 pandemic.[9] The strict nonpharmaceutical interventions in 2020 to combat the COVID-19 pandemic changed the RSV circulation pattern and resulted in a delay in the annual RSV outbreak in several countries.[2,10]

Since the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spread out in December 2019, the respiratory virus infection, including RSV, has remained low in Taiwan due to universal masking and social distancing. However, we observed a surge in pediatric RSV infections in southern Taiwan since September 2020. In this study, we investigated the clinical and molecular epidemiology of pediatric RSV infection before and during the COVID-19 pandemic.

Materials and methods

Study design and patient definition

This is a retrospective study of pediatric patients with virologically confirmed RSV infection from the inpatient, outpatient, or emergency departments at the National Cheng Kung University Hospital (NCKUH), a national university-affiliated medical center in southern Taiwan. Patients less than 18 years old diagnosed with RSV infection between January 2016 to December 2020 were enrolled. During the 2020 outbreak, all hospitalized children with respiratory symptoms were tested for respiratory viruses, either using an RSV antigen test, a viral culture, or both. There was a total of 554 children hospitalized with respiratory symptoms in 2020 and 738 respiratory specimens tested for respiratory viruses. RSV infection was defined as a positive RSV antigen test and/or RSV isolated from a viral culture using respiratory specimens, including throat swabs or nasopharyngeal aspirates. According to our clinical practice, respiratory specimens obtained from nasopharyngeal aspirates were performed by nurses or nurse practitioners among hospitalized children during the daytime. Patients from the emergency department, outpatient department, or admission during duty hours were tested for respiratory viruses using throat swab performed by attending physicians or resident physicians. A list of patients testing positive for RSV was retrieved from the hospital-based electronic medical database, as well as demographic and epidemiological data, clinical presentation, diagnosis, treatment course, clinical outcomes, and laboratory findings for those patients. However, patients without complete clinical information available for analysis were excluded. The NCKUH clinical virology laboratory, a contracted laboratory of the nationwide viral surveillance system of the Centers for Disease Control (CDC) in Taiwan, is responsible for viral surveillance in southern Taiwan. The RSV positive rate was defined as the number of RSV isolates divided by the total number of respiratory specimens and multiplied by 100. Acute bronchiolitis was diagnosed in children presenting with expiratory wheezing on auscultation and radiological evidence of pulmonary hyperinflation, peri-bronchial infiltrates, or diffuse interstitial markings. Bronchopneumonia was characterized in patients with chest radiological findings suggestive of suppurative peribronchiolar inflammation and patches involving multiple lobes of the lung. Finally, lobar pneumonia was defined as pneumonia localized to one or more lobes of the lung in which the affected lobe or lobes are completely

consolidated. This study was approved by the Institutional Review Board (IRB) of the NCKUH (No. A-ER-110-199).

Specimen collection and viral identification

Respiratory specimens were collected into viral transport medium at 2～8°C and then immediately transported to the NCKUH clinical virology laboratory. Respiratory specimens in viral transport medium with antibiotics were inoculated into several cell lines, including human lung carcinoma (A549), rhabdomyosarcoma (RD), and Madin—Darby canine kidney (MDCK) cells. These viral culture tubes were incubated in a 35 °C, CO_2 incubator and examined for cytopathic effects (CPE) daily for 10—14 days. Viral identification was done through immunofluorescent staining with virus-specific monoclonal antibody (D^3 Ultra 8 DFA Respiratory Virus Screening & Identification Kit, Quidel, San Diego, CA, USA). Twenty-one respiratory specimens (14 viral isolates and 7 throat swab samples) from 14 patients requiring intensive care were collected for further phylogenetic analysis. We randomly selected age-matched inpatients (only required general ward hospitalization) in a 1:2 ratio to manage more RSV isolates representing mild infection. In the end, a total of 63 RSV isolates were sequenced.

Phylogenetic analysis

To identify the genotype and phylogenetic relationship of RSV in Taiwan, a total of 129 RSV F gene and 126 RSV G gene sequences from GenBank were also included for phylogenetic analysis (Supplementary Table 1). The nucleotide (nt) sequence involved in the phylogenetic analysis corresponded to 112—1587 nt (238—529 amino acid) of the F gene coding region and 325—891 nt (109—308 amino acid) of the G gene coding region, respectively, from the RSV-A reference strain M11486. Nucleotide sequences were aligned based on the deduced amino acid sequences using the MEGA5 software.[11] The maximum-likelihood (ML) tree was reconstructed with the best-fit evolutionary model and 1000 bootstrap replications were also performed using MEGA 5.[11]

Statistical analysis

All statistical analyses were performed using commercially available statistical software (IBM SPSS Statistics for Windows, Version 25.0). Descriptive analyses of numerical variables are presented as mean and standardized deviation (mean ± SD), and categorical variables are presented as frequency and percentage. Continuous data were analyzed using Student's t-test; categorical data were compared using the Fisher's exact test. All tests were two-tailed. A p-value < 0.05 was considered statistically significant.

Results

Epidemiology of RSV infection during 2016—2020

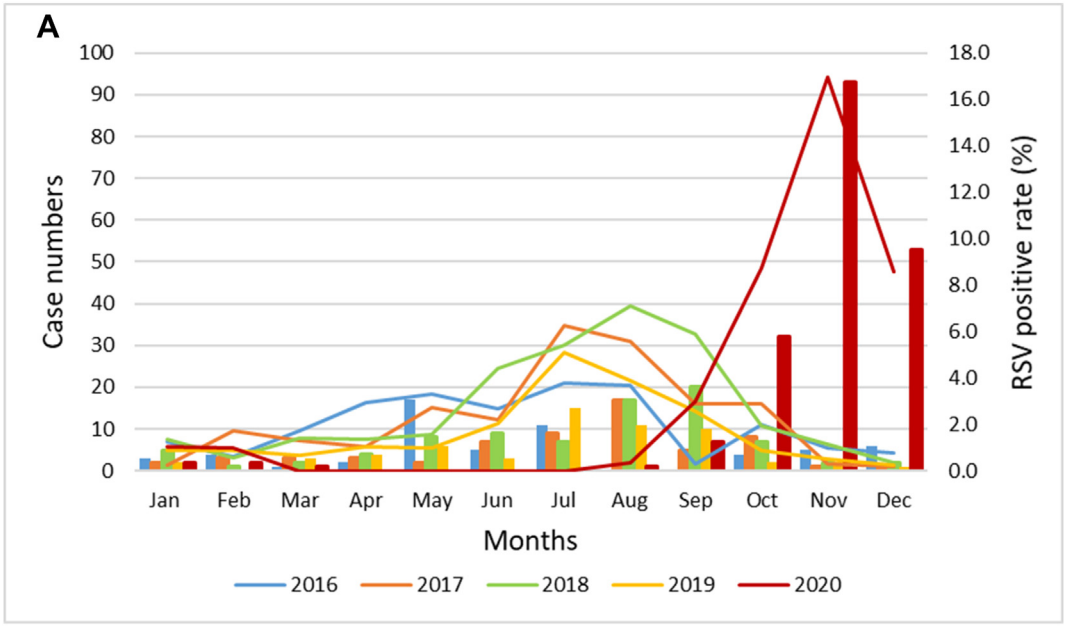
The monthly distribution of RSV infections throughout the study period is displayed in Fig. 1A. From 2016 to 2019, RSV cases displayed significant seasonal distribution, with consistent peaks in the summer to mid-autumn (average 14 cases in spring, 28 in summer, 17 in autumn, 8 in winter). In 2020, RSV cases remained low until late summer, and there were no cases from April through July. However, a significant surge was observed after September 2020 and lasting through December, with a peak of 93 cases in November. There were a median of 59 annual RSV cases from 2016 to 2020, but there were 186 cases in 2020. The RSV positive rate in 2020 was 0.0% from April to July, 3.0% in September, 8.7% in October, 17.0% in November, and 8.6% in December. Compared with the nationwide respiratory viral surveillance database provided by the Taiwan CDC, the RSV positive rate was 0.0% from April to July, 3.0% in September, 7.7% in October, 16.0% in November, and 8.4% in December in 2020. The trend of RSV cases detected in our hospital parallels the rise of RSV cases in the CDC respiratory viral surveillance database,[12] indicating a national epidemic (Fig. 1B).

Demographics and clinical characteristics of RSV cases

Overall, a total of 442 RSV cases were enrolled during the study period. Patient demographics and clinical findings are summarized in Table 1. Around 26.5% of patients had systemic underlying diseases, including premature birth (16.3%), chronic lung disease (3.4%), congenital heart disease (3.2%), neurological disorders (4.1%), being immunocompromised (1.6%), and asthma (1.4%). The most common presentations were cough (98.4%), rhinorrhea (93.0%), and fever (81.9%), followed by crackles (68.1%), wheezing, (55.7%), retraction (43.7%), dyspnea (38.0%), hypoxemia (32.1%), vomiting (15.6%), diarrhea (13.6%), skin rash (3.8%), and abdominal pain (2.3%). The most common clinical diagnoses were acute bronchiolitis (53.6%) and bronchopneumonia (42.5%). A total of 371 (83.9%) cases were admitted to the hospital, 48 (12.9%) cases required intensive care, and 35 (7.9%) cases needed ventilator support. The mean hospitalization period was 4.3 days.

To characterize the clinical presentation of RSV infection in 2020, we divided the study period into two parts, with study period one from January 2016 to December 2019 (256 cases) and study period two from January 2020 to December 2020 (186 cases). There were no significant differences in gender, contact history, and underlying disease between two study periods. However, compared with 2016—2019, the mean age of the RSV-infected children in 2020 was significantly older (2.3 ± 2.2-year-old vs. 1.0 ± 1.0-year-old, $p < 0.001$). Most patients in study period one were younger than 6 months old, and up to 57.8% cases were younger than 1-year-old (Fig. 2). In contrast, up to 50% of cases in study period two were above 2 years old, with only 24.2% younger than 1 year.

The hospitalization rate and hospital stay were significantly lower in study period two compared with study period one (hospitalization rate: 74.7% vs. 90.6%; hospital stay: 3.4 ± 4.5 vs. 4.9 ± 5.7 days, all p value < 0.05). More cases were diagnosed with acute bronchiolitis in study period one (66.8% in 2016—2019 vs. 35.5% in 2020), while more cases were diagnosed with bronchopneumonia in



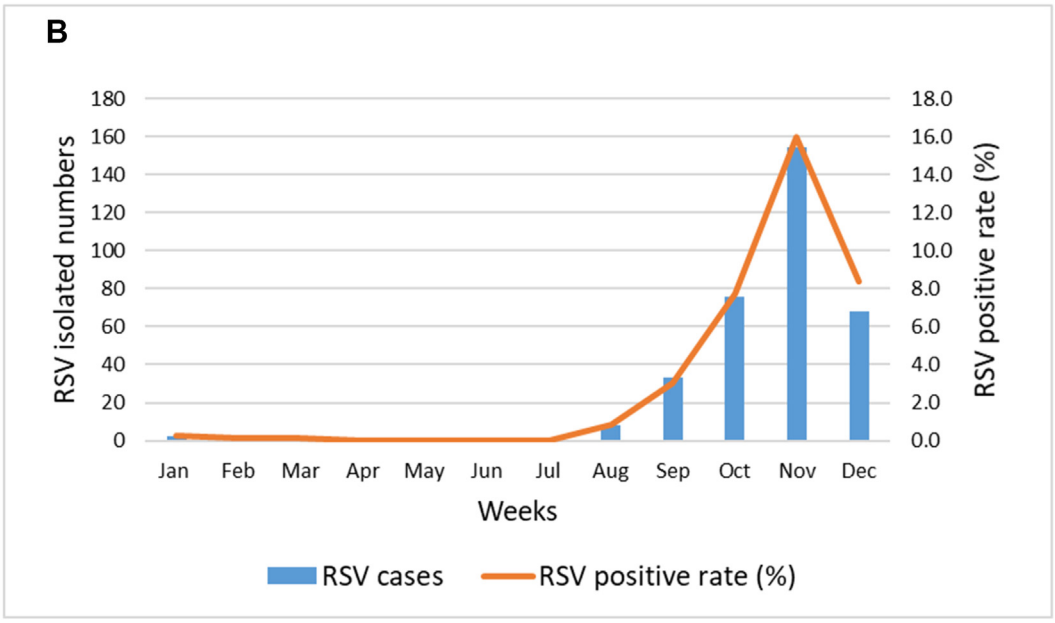


Figure 1. (A) Monthly distribution of the pediatric RSV infections during 2016—2020. (B) RSV cases and positive rate by month from Taiwan CDC national respiratory surveillance database in 2020.

study period two (57.5% in 2020 vs. 31.6% in 2016—2019, all p value < 0.05). Moreover, patients in study period two presented with a lower white blood count, lymphocyte count, and platelet count, and higher segment count compared to patients in study period one (all $p < 0.05$).

Table 2 summarizes the disease severity of RSV infection according to clinical parameters, including hospital stay, ICU admission rates, need for oxygen use and/or ventilator support, antibiotics use, hypoxemia, retraction, and dyspnea. We conducted further multivariate analysis by controlling for age, gender, and underlying diseases to reduce confounding factors. The results revealed that patients in study period two had longer duration of fever (3.7 ± 2.1 days vs. 2.7 ± 2.3 days, p value $= 0.025$), but had a shorter hospital stay, less oxygen use, and less respiratory distress including hypoxemia, retraction, and dyspnea, compared to study period one (all $p < 0.05$).

RSV strains isolated in 2020

Of total 738 respiratory specimens tested among hospitalized children in 2020, 191 were found to be positive for RSV antigen test and/or RSV isolated from viral culture. After excluding 52 duplicate respiratory specimens obtained from the same patients, there were a total of 139 virologically-confirmed RSV infections hospitalized children in 2020. Of the total 186 RSV-confirmed cases form hospitalization,

Table 1 Demographic and clinical characteristics of children with RSV infection during 2016–2020.

	2016	2017	2018	2019	2016–2019	2020
Number of RSV cases	58	57	82	59	256	186
Highest monthly RSV cases	May	Aug	Sep	Jul	Aug	Nov
Age (years), mean ± SD	0.8 ± 0.8	1.2 ± 1.3	1.1 ± 0.9	1.0 ± 0.9	1.0 ± 1.0	2.3 ± 2.2[a]
<6 months, no. (%)	30 (51.7)	27 (47.3)	32 (39.0)	26 (44.0)	115 (44.9)	19 (10.2)
6 months - 1 year, no. (%)	9 (15.5)	5 (8.8)	10 (12.1)	9 (15.3)	33 (12.9)	26 (14.0)
1–2 years, no. (%)	12 (20.7)	16 (28.0)	28 (34.1)	17 (28.8)	73 (28.5)	48 (25.8)
2–3 years, no. (%)	4 (6.9)	8 (14.0)	11 (13.4)	4 (6.8)	27 (10.5)	42 (22.6)
3–4 years, no. (%)	1 (1.7)	0 (0.0)	0 (0.0)	3 (5.1)	4 (1.6)	24 (12.9)
4–5 years, no. (%)	1 (1.7)	0 (0.0)	1 (1.2)	0 (0.0)	2 (0.8)	17 (9.1)
>5 years, no. (%)	1 (1.7)	1 (1.8)	0 (0.0)	0 (0.0)	2 (0.8)	10 (5.4)
Male sex, no. (%)	36 (62)	42 (73.7)	46 (56.1)	34 (57.6)	158 (61.7)	102 (54.8)
Contact history, no. (%)	33 (56.9)	31 (54.4)	47 (57.3)	37 (62.7)	148 (57.8)	92 (49.5)
Underlying diseases, no. (%)	26 (44.8)	13 (22.8)	20 (24.3)	16 (27.1)	75 (29.3)	42 (22.6)
Immunocompromised	1 (1.7)	1 (1.8)	1 (1.2)	0 (0.0)	3 (1.2)	4 (2.1)
Prematurity	21 (36.2)	7 (12.3)	9 (11.0)	10 (16.9)	47 (18.4)	25 (13.4)
Congenital heart disease	3 (5.2)	3 (5.3)	1 (1.2)	2 (3.4)	9 (3.5)	5 (2.7)
Chronic lung disease	5 (8.6)	3 (5.3)	1 (1.2)	1 (1.7)	10 (3.9)	5 (2.7)
Asthma	2 (3.4)	0 (0.0)	1 (1.2)	0 (0.0)	3 (1.2)	3 (1.6)
Neurological disorders	2 (3.4)	2 (3.5)	6 (7.3)	4 (6.8)	14 (5.5)	4 (2.1)
Others	1 (1.7)	0 (0.0)	3 (3.7)	3 (5.1)	7 (2.7)	2 (1.1)
Fever days (mean ± SD)	2.5 ± 2.2	2.5 ± 2.5	2.9 ± 2.3	2.7 ± 2.1	2.7 ± 2.3	3.7 ± 2.1[a]
Hospitalization, no. (%)	54 (93.1)	51 (89.5)	74 (90.2)	53 (89.8)	232 (90.6)	139 (74.7)[a]
Hospitalization days (mean ± SD)	4.4 ± 3.2	4.7 ± 5.7	5.2 ± 7.4	5.1 ± 4.8	4.9 ± 5.7	3.4 ± 4.5[a]
ICU admission, no. (%)	7 (12)	5 (8.8)	12 (14.6)	10 (16.9)	34 (13.3)	14 (7.5)
Diagnosis						
Bronchiolitis, no. (%)	42 (72.4)	39 (68.4)	52 (63.4)	38 (64.4)	171 (66.8)	66 (35.5)[a]
Bronchopneumonia, no. (%)	16 (27.6)	15 (26.3)	30 (36.6)	20 (33.9)	81 (31.6)	107 (57.5)[a]
Croup, no. (%)	0 (0)	1 (1.7)	1 (1.2)	0 (0)	2 (0.8)	2 (1.1)
Lobar pneumonia, no. (%)	0 (0)	0 (0)	2 (2.4)	0 (0)	2 (0.8)	0 (0)
Acute nasopharyngitis, no. (%)	0 (0)	2 (3.5)	0 (0)	1 (1.7)	3 (1.2)	11 (5.9)
Laboratory data						
WBC ($\times 10^9$/L)	9.6 ± 3.7	10.1 ± 5.0	10.2 ± 4.1	9.1 ± 3.4	9.8 ± 4.1	8.9 ± 4.6[a]
Segment (%)	35.2 ± 18.5	39.3 ± 18.5	34.5 ± 16.0	34.8 ± 11.8	35.8 ± 16.4	44.0 ± 18.7[a]
Band (%)	2.5 ± 4.0	4.2 ± 6.0	4.8 ± 7.1	5.9 ± 9.4	4.4 ± 7.0	5.4 ± 5.5
Lymphocyte (%)	46.7 ± 18.9	43.5 ± 19.7	47.0 ± 16.5	46.4 ± 15.9	46.0 ± 17.6	37.9 ± 18.3[a]
Platelet ($\times 10^9$/L)	328 ± 111	328 ± 131	341 ± 134	338 ± 114	334 ± 123	283 ± 106[a]
CRP (mg/L)	19.8 ± 48.6	11.9 ± 23.6	16.2 ± 23.6	9.2 ± 12.1	14.5 ± 29.7	12.0 ± 22.4

[a] Statistically significant difference ($p < 0.05$) comparing variable between 2016-2019 and 2020.
Abbreviations: mean ± SD: mean ± standard deviation, ICU: intensive care unit, WBC: white blood count, CRP: C-reactive protein.

emergency department, and outpatient department in 2020, 181 had a positive RSV antigen test, 116 had RSV isolated from a viral culture, and 111 had both. Among them, 63 samples from age-matched inpatients requiring either intensive or general care were sequenced for phylogenetic analysis. Out of those, 46 were confirmed as RSV positive and positive for the F gene using polymerase chain reaction (PCR). 32 F-gene positive samples also tested positive with PCR for genotype A of the G gene (RSV-A). All PCR products were sequenced and confirmed to belong to RSV genotype A.

After excluding sequences containing frameshift mutations of either the F gene or G gene, a total of 40 F gene sequences and 27 G gene sequences from this study were included in the phylogenetic analysis (Supplementary Table 1). According to the ML tree of G gene and F gene, all sequences from this study belonged to RSV-A subtype ON1, including those in which the G gene failed to amplify (Fig. 3). The tree topology of the G gene tree was consistent with the F gene tree, and both showed that the ON1 in Taiwan in 2020 was divided into two bootstrap value supported clades. We tentatively named the major clade with a higher sample size as TW20A and the minor clade with a lower sample size as TW20B (Fig. 3). TW20A and TW20B were further clustered with some recently identified ON1 strains that share a common ancestor. We tentatively named this monophyletic group as ON1s (Fig. 3). According to the G gene tree, TW20A and TW20B were not from the ON1 that was prevalent in Taiwan from 2013 to 2016, as found in the previous study.[13] We hypothesize that both TW20A and TW20B may have evolved independently in other regions, was introduced to Taiwan, and then replaced the previously dominant strain (Fig. 3A). Although the Taiwan strain of the F gene was less available and no relationship with the pre-2016 Taiwan strain could be

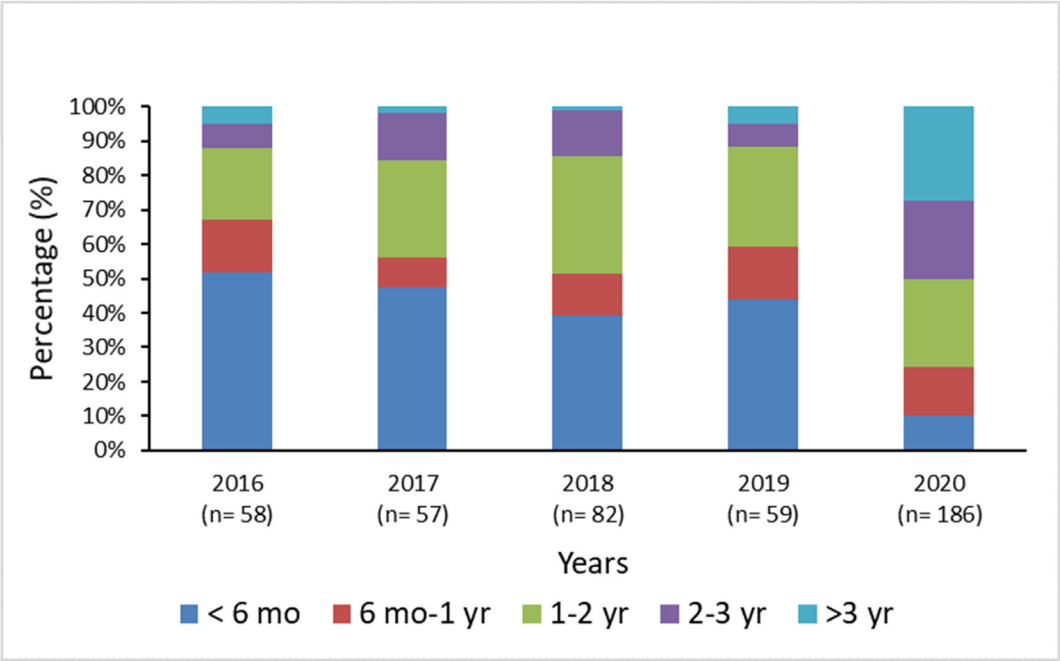


Figure 2. The age distribution of RSV infected children during 2016—2020.

Table 2 The univariate and multivariate analysis of clinical variables relating to disease severity between 2016-2019 and 2020.

	Univariate analysis			Multivariate analysis	
	Study period				
	2016—2019 (n = 256)	2020 (n = 186)	P-value	Adjusted OR (95% CI)[b]	P-value
Fever days, mean ± SD	2.7 ± 2.3	3.7 ± 2.1	**<0.001***	1.68 (1.07—2.64)	**0.025***
Hospitalization days, mean ± SD	4.9 ± 5.7	3.4 ± 4.5	**0.004***	0.58 (0.38—0.90)	**0.015***
ICU admission, no. (%)	34 (13.3%)	14 (7.5%)	0.055	0.58 (0.28—1.20)	0.14
Oxygen use, no. (%)	147 (57.4%)	72 (38.7%)	**<0.001***	0.46 (0.30—0.70)	**<0.001***
Ventilator use, no. (%)	27 (10.5%)	8 (4.3%)	**0.016***	0.47 (0.20—1.15)	0.097
Hypoxemia[a],no. (%)	101 (39.5%)	41 (22.0%)	**<0.001***	0.45 (0.28—0.73)	**0.001***
Retraction, no. (%)	138 (53.9%)	55 (29.6%)	**<0.001***	0.36 (0.23—0.55)	**<0.001***
Dyspnea, no. (%)	116 (45.3%)	52 (28.0%)	**<0.001***	0.54 (0.34—0.86)	**0.009***
Antibiotics use, no. (%)	126 (49.2%)	102 (54.8%)	0.243	1.24 (0.82—1.87)	0.315

[a] Peripheral oxygen saturation of <95% at sea level.
[b] Adjusted by age, gender, and underlying diseases using multivariate logistic regression.
Abbreviations: OR: odds ratio; CI: confidence interval.
*p values in bold font indicate statistical significance.

found, the clade of TW20A and TW20B was consistent with the results obtained from the G gene (Fig. 3B).

Discussion

We observed a surge in pediatric RSV infections in southern Taiwan between September and December 2020, which paralleled the nationwide RSV epidemiology from the Taiwan CDC surveillance system. Patients in the 2020 outbreak are older and presented with more prolonged febrile illness compared to previous years. In the 2020 outbreak, bronchopneumonia replaced acute bronchiolitis as the most common diagnosis, but patients displayed a more attenuated clinical course with lower hospitalization rates, a shorter hospital stay, less oxygen use, and less respiratory distress. RSV-A subtype ON1 was the major genotype identified in 2020.

Unlike countries in temperate zones, there is no dominant RSV season in Taiwan. Previous studies found that RSV infection usually peaks in spring and fall in Taiwan.[7,8,14] Our study demonstrated that RSV cases peaked in summer in southern Taiwan during the non-outbreak period (2016—2019), while an unusual rise in RSV cases was

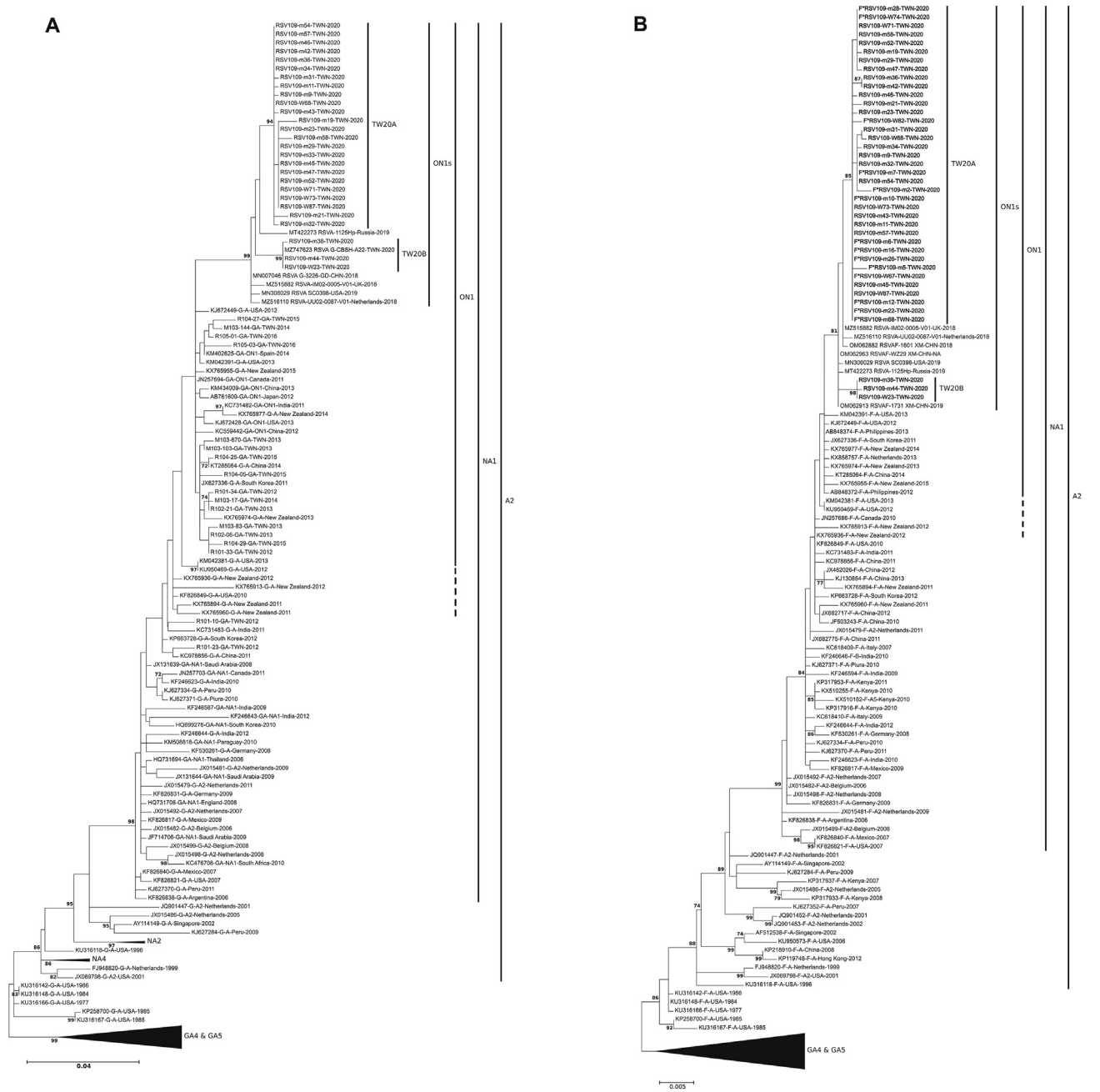


Figure 3. Unrooted maximum-likelihood tree of RSV-A G gene and F gene. (A) The maximum-likelihood (ML) tree of G gene. (B) ML tree of F gene. Bootstrap values are labeled at branches. Branches with a bootstrap value equal or above 70 are considered as strong supported ($p < 0.05$). Sequences from this study are labeled in bold. The scale bar at the left bottom indicates evolutionary distance in unit of substitutions per site. The lines at the right site indicate major RSV-A subtypes. The dash lines indicated an intermediated region that included both with and without 72-bp duplication strains that descripted in the previous study.[13] F gene sequences that G gene failed to amplify were labeled with F* in F gene tree.

observed in the winter of 2020. SARS-CoV-2 emerged at the end of 2019 and spread rapidly, with the World Health Organization (WHO) declaring it a global pandemic on March 11, 2020. Since then, social activities have been restricted worldwide, and infection control measures, including handwashing, mask wearing, and social distancing, have been strengthened. In addition to interrupting the spread of COVID-19, these measures reduced the worldwide prevalence of other respiratory virus infections, such as influenza and RSV.[15–17] Taiwan CDC's respiratory viral surveillance database also showed similar epidemiological trends before this RSV outbreak. The annual case numbers in 2020 were above 2.9-fold the median seasonal peak during 2016–2019. A recent study from Australia demonstrated that RSV activity increased from September 2020 and exceeded the median seasonal peak.[18] Another study

from New York City reflected similar findings.[2] These may be due to relaxed physical distancing recommendations and opening of interstate borders. Although there was no dominant RSV season in Taiwan and our strict non-pharmaceutical interventions against COVID-19 had not been released during the study period, we observed a surge in pediatric RSV infections since September 2020. In contrast, the spread of other annually circulating viruses, such as influenza and enterovirus, were significantly reduced.[19,20] This outbreak may be attributed to viral factors of this novel RSV-A genotype ON1 variants.

Infants with RSV infections develop more severe disease because of their immature immune system or because they are undergoing a primary infection. The decline in the risk of RSV-associated lower respiratory tract infection with increasing age has conventionally been attributed to accumulated immunity following previous RSV exposure, ontogeny of the immune system, and physiological changes, such as larger airways.[21] In school-aged children, humoral immunity wanes over time and reinfection during subsequent seasons is often seen.[22] A recent study from western Australia found a resurgence of RSV in children following the reduction of COVID-19 related public health measures. They speculated that the increase in median age and the rise in numbers may be due to the expanded cohort of RSV-naïve patients, including an increased number of older children, coupled with waning population immunity.[18,23] The older RSV infected children in 2020 may contribute to decrease in disease severity and the proportion of acute bronchiolitis.

A novel RSV-A genotype ON1, with a 72-nucleotide duplication in the C-terminal region of the G protein, was first reported in Ontario, Canada in December 2010.[24] After this report, several countries, including Italy, Japan, Germany, South Africa, Thailand, Korea, China, Croatia, Malaysia, and India, submitted ON1 sequences to GenBank, suggested that the ON1 genotype was transmitted globally and quickly.[25—33] Numerous variants of the ON1 genotype have been reported worldwide, with various mutations and amino acid substitutions. The ON1-1.1 variant has six amino acid substitutions, including T200P, P215L, N255D, S275N, N279I, and E295V. The ON1-1.2 variant has L274P and L298P substitutions. The ON1-1.3 variant is characterized by the distinctive I243S and E262K substitutions, while retaining

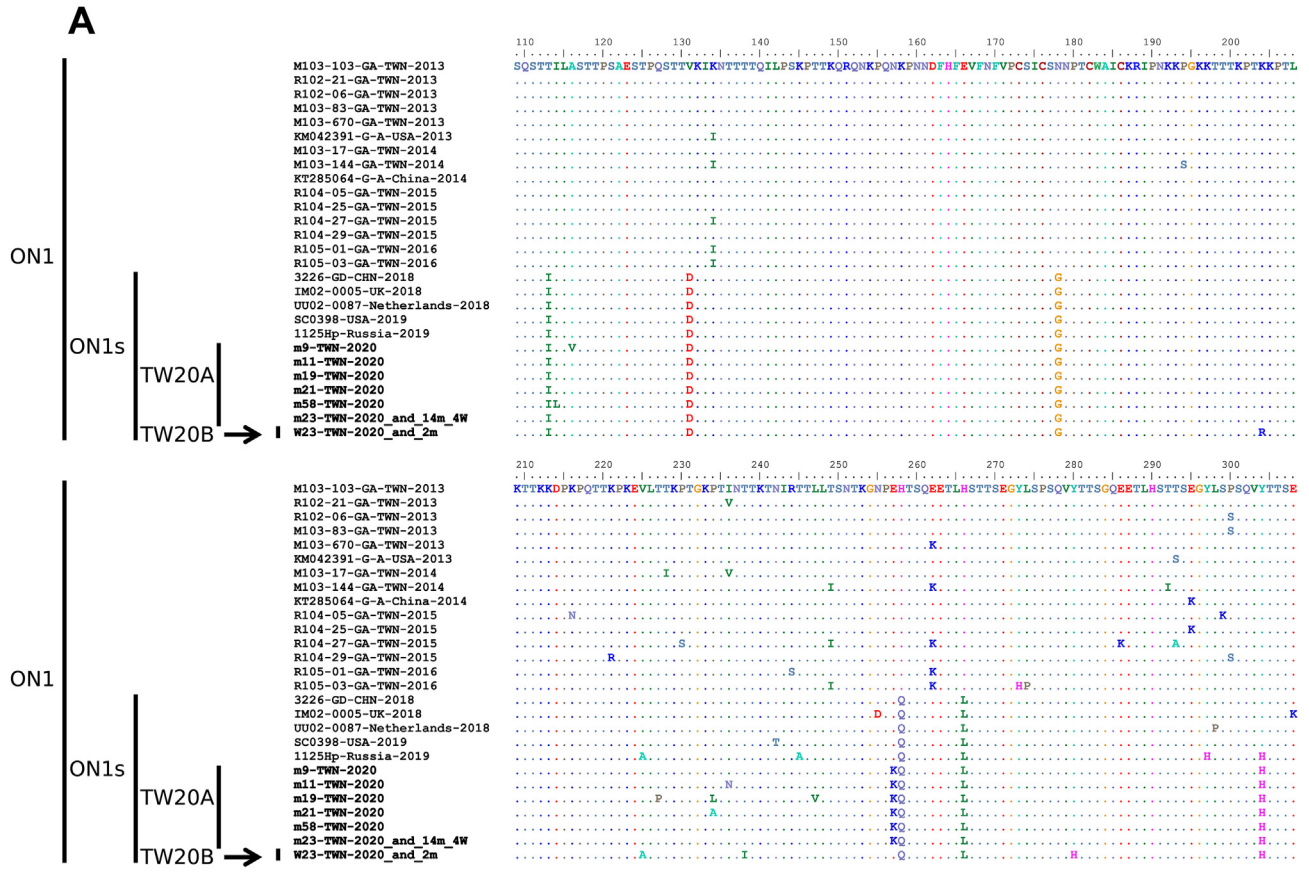


Figure 4. Alignment of the G gene and F gene deduced amino acids in Taiwan. (A) The alignment of amino acid position 109—308 of G protein and (B) 238—529 of F protein was shown. Alignment of G gene amino acid sequences in this study were compared with representative ON1 strains that prevalent 2013 to 2016 in Taiwan; and/or KM042391 and KT285064 which are most similar to ON1 prevalent in Taiwan in 2013. Sequences from this study are labeled in bold. The lines at the left site indicate major RSV-A subtypes and clades (according to Fig. 3). Only one of the identical sequences is retained, and the retrieval source is annotated (numbers with m and/or numbers with W) after the retained sequence. F gene sequences that G gene failed to amplify were labeled with F* in F protein alignment.

L274P and L298P in most isolates. Finally, the ON1-1.4 variant has the I/T136T and a unique P206Q substitution that distinguished it from the other ON1 lineages.[34,35]

According to the previous study, ON1 became an exclusive strain after 2013 and its evolution continued until 2016.[13] The ON1 outbreak in Taiwan in 2020, however, evolved independently in a different region from the ON1 prevalent in Taiwan until 2016. The existence of the Russian strain MT422273 between TW20A and TW20B suggested that TW20A, TW20B, and the Russian strain shared a common ancestor. TW20A and TW20B might have evolved outside of Taiwan and introduced into Taiwan at similar, separate point in time.

The RSV-infected children in 2020 displayed significantly decreased disease severity, including lower hospitalization rates, shorter hospital stays, less need for oxygen supplementation, and less severe clinical manifestations such as hypoxemia, retraction, and dyspnea. We compared the amino acid sequences of G gene samples from Taiwan in 2020 with those of ON1 from 2013 to 2016 to investigate the possibility of 2020 outbreak unique substitutions. The mutations that evolved in the Taiwan ON1 strain between 2013 and 2016, including K134I, T249I and E262K, could not be found in the 2020 strains. The substitutions T113I, V131D, N178G, H258Q, and H266L presented in the monophyletic group ON1s containing TW20A and TW20B, as well as other recent RSV strains from other regions (Fig. 4A). The Y304H substitution occurred only in the TW20A and TW20B strains, as well as in the Russian strain (MT422273). The unique E257K substitution existed only in the TW20A clade and the substitutions K204R, V225A, T238I, Y280H existed only in the TW20B clade.

Since the RSV F gene sequences were less available in Taiwan, KM042391 and KT285064, which were most similar to the ON1 prevalent in Taiwan in 2013 based on the G gene evolutionary tree, were selected for comparison with samples from 2020. H514N substitution was found in the TW20A clade but not present in other ON1 clades (Fig. 4B).

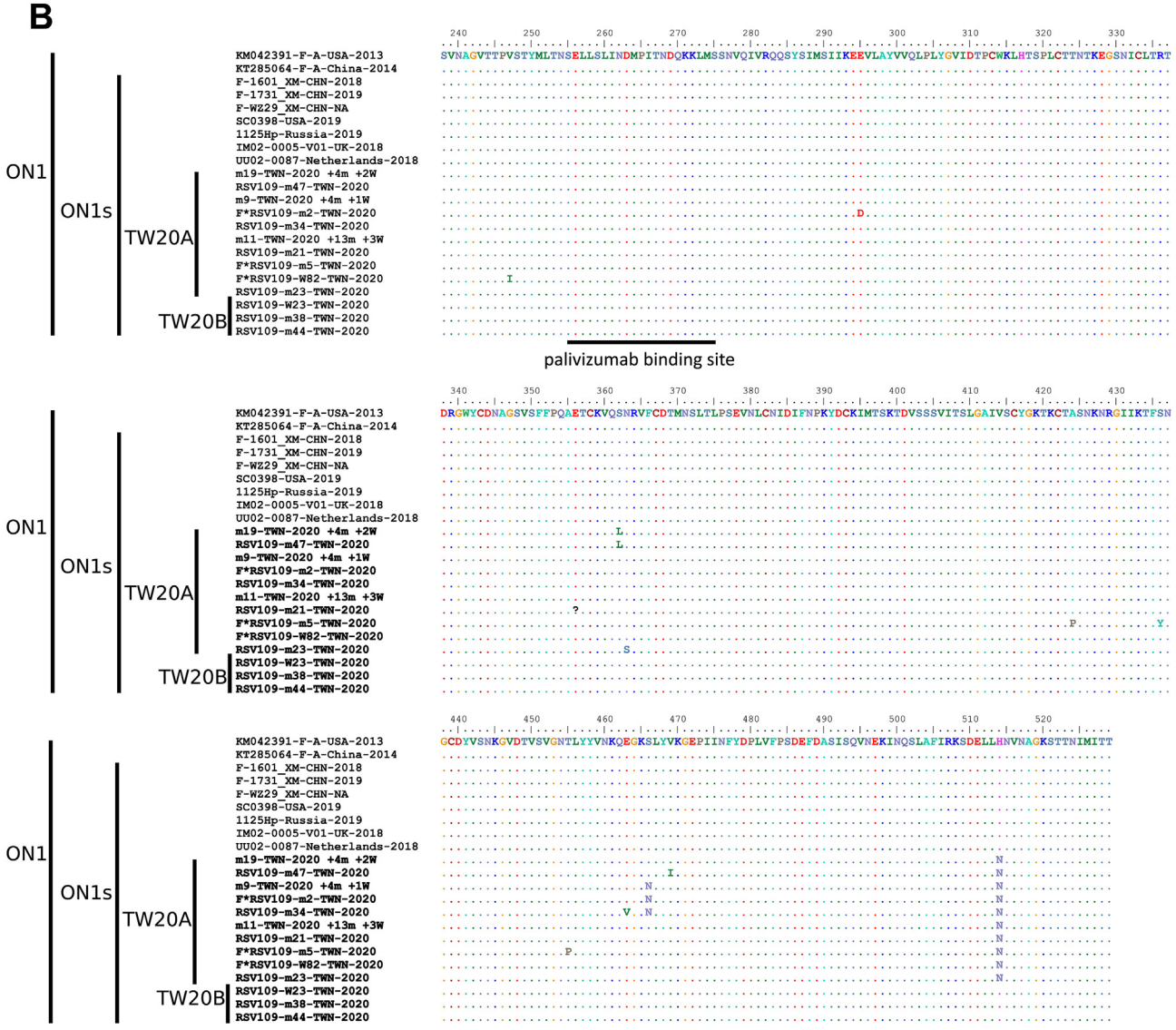


Figure 4. *(Continued).*

The S362L substitution was specific to the m19 clade (m19, m28, m29, m47, m52, m58, W71, and W74), and the S466N substitution was specific to the m9 clade (m2, m7, m9, m31, m32, m34, m54, and W68), of TW20A. The antigenic sites corresponding to amino acids 255—275 of the F protein did not harbor any amino acid substitution in all samples of both the TW20A and TW20B clades.

A recent retrospective study in Taiwan that enrolled children <5 years old hospitalized with culture-confirmed RSV infections between 2018 and 2020 showed similar results. In this study, six amino acid substitutions (T113I, V131D, N178G, H258Q, H266L, and Y304H) occurred in 2019. The E257K substitution in the G protein and H514N in the F protein were unique substitutions and first emerged in 2020, similar to our findings. However, in their study, the ON1 variant in 2020 was independently associated with an O_2 saturation <94% during hospitalization, while our patients in 2020 had less respiratory distress, including hypoxemia, retraction, and dyspnea.[36] In their study, the median age of RSV-positive patients in 2020 was 19.78 ± 14.28 months old and multivariate analysis revealed that age was also associated with an O2 saturation <94%. In the current study, we had a higher number of older RSV-infected children, who tended to have less severe disease. It's likely that for the discrepancy in results between the two studies.

The severity of RSV infections is multifactorial and depends both on host and viral factors. Several studies have addressed this issue; however, there are conflicting results regarding ON1-related disease severity. Studies in northern Italy and Cyprus both demonstrated that ON1-infected children had a lower incidence of lower respiratory tract infections, milder illness, and less frequent hospitalization than genotype NA1, GA2, or BA.[37,38] In contrast, a study in Vietnam found that ON1 was associated with an increased risk of respiratory clinical signs or symptoms and disease severity in children under 5 years old.[39] Another study demonstrated that the divergence of ON1 strains was associated with an increase in bronchiolitis clinical severity.[34] Also, some studies found no significant difference in clinical features between ON1 and non-ON1 infections.[40] Whether an RSV genotype or variant affects disease severity warrants further exploration, as the current study results were inconclusive. Nevertheless, these characteristic amino acid substitutions and the change of new glycosylation sites suggest these could be associated with modified virus behavior.

There were several limitations of this study. First, this was a hospital-based retrospective study. We retrospectively enrolled pediatric patients visiting NCKUH with virologically confirmed RSV infection. In clinical practice, patients with newly onset respiratory symptoms suggestive of acute viral illness might warrant further microbiological investigation. However, during 2016—2019 non-outbreak period, not all children with respiratory symptoms were tested for respiratory viruses. The virological tests are subject to the clinician's judgement, which might lead to potential selection bias. Second, our hospital is a tertiary medical center and most of our patients present with a higher disease severity than regional hospitals or general practitioners. Our cases might represent only part of the RSV infections and be more predominantly severe cases. Nevertheless, we have surveillance data to observe the epidemiology of RSV infection in the most recent five years. The Taiwan CDC national respiratory surveillance database has monitored inpatient and outpatient visits for patients with acute influenza-like infections. The trend of RSV cases detected in our hospital parallels the rise of RSV cases in the CDC respiratory viral surveillance database. This echoes the epidemiological association of community outbreak and the surge of moderate to severe cases presenting to our hospital.

In conclusion, we observed an unexpected RSV surge during September and December 2020 in southern Taiwan during the COVID-19 pandemic. Despite there being a 2.9-fold increase of annual case numbers in 2020, the clinical manifestations and severity of this RSV epidemic outbreak were less severe than in 2016—2019. Patients in the 2020 outbreak were older and bronchopneumonia was the most common diagnosis. We identified the novel RSV-A subtype ON1 variant as responsible for this outbreak. The waning herd immunity to RSV might have also facilitated the spread of this variant, but the accumulated naïve population who are more immunologically mature due to older age might have decreased the disease severity. Continuous epidemiological monitoring is needed for provision of better supportive care and prompt diagnosis.

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Authors' contributions

T-YL, C-FS and C-CL contributed to the concept and design of the study; T-YL, C-YK, and H-PT acquired the data and clinical materials for analysis. T-YL, and HC analyzed and interpreted the data and prepared figures and tables. T-YL and C-YK carried out the statistical analysis. T-YL, HC, and C-FS prepared the first draft of the manuscript. HC, H-PT, J-RW, C-FS and C-CL revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2022.08.015.