**Epidemiology and genetic evolutionary analysis of influenza virus among children in Hainan Island, China, 2021 – 2023**

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**Abstract**

**Background** During the COVID-19 pandemic, we continuously monitored the epidemiology of influenza virus among pediatric patients from January 2021 to December 2023 in Hainan Island, China.

**Methods** In this study, we collected 54,974 nasopharyngeal swab samples for IAV testing and 53,151 samples for IBV testing from pediatric outpatients. Additionally, we also collected 19,687 nasopharyngeal swab samples from pediatric inpatients for IAV and IBV testing. Outpatient samples were screened for IVs infection by the colloidal gold method. Targeted Next-Generation Sequencing (tNGS) was used to detect Influenza virus infections in inpatients. Influenza virus types were identified by analyzing the HA/NA partial regions.

**Results** The findings revealed a decrease in the infection rate of IBV over the specified period, while the infection rate of IAV exhibited a rising trend. Additionally, B/Victoria lineage was the dominant epidemic strain in 2021, while the epidemic strains in 2022 and 2023 underwent a dynamic transformation from A/H3N2 to A/H1N1. Phylogenetic analysis revealed close relationships among the circulating strains. Nonetheless, because the sample size is limited, additional research is required.

**Conclusion**

Our findings suggest that the predominant types of influenza viruses in the pediatric population are dynamically changing, and non-pharmaceutical intervention measures can significantly impact the infection rate of influenza.

**Keywords** Influenza virus, COVID-19, Epidemiology, Children, Hainan Island,

**1 Introduction**

The Influenza virus (IVs), belonging to the Orthomyxoviridae family, is an RNA virus that can induce acute respiratory infections, exhibiting both seasonal and epidemic attributes[1]. To date, circulating influenza virus can be categorized into four overarching classifications: influenza A virus (IAV)，influenza B virus (IBV)，influenza C virus (ICV) and influenza D virus (IDV)[2] . Among these, IAV and IBV are mainly responsible for impact on public health, with IAV provoking more severe clinical diseases and seasonal epidemics in humans[3, 4]. Influenza epidemics occur worldwide annually, is estimated to result in more than 3 to 5 million infections and approximately 291,000 to 646,000 deaths each year[5, 6]. It is noteworthy that infections among children make a substantial contribution to the global burden of disease and mortality during the influenza season. Each year, millions of children worldwide experience influenza infections, and it is estimated that between 9,243 and 105,690 respiratory deaths annually are attributed to seasonal influenza among children under 5 years of age [5, 7].

At the end of 2019, the coronavirus disease 2019 (COVID-19) broke out suddenly and spread rapidly across the world. To prevent and control the epidemic of COVID-19, many countries have adopted strong non-pharmaceutical intervention (NPI) measures, which has changed the lifestyles of majority of people. Since then, Chinese government has always adhered dynamic zero-COVID policy and implemented a range of measures aimed at curtailing the further dissemination of the virus, including lockdowns, closures of schools, and social isolation. During the COVID-19 pandemic, although the influenza diagnosis rate varies in space and time in different countries, the significant decline compared with the pre-epidemic level is global[8]. The study found that countries with zero-COVID experienced the significant decline, followed by most temperate countries with high COVID-19 incidence and social restrictions, while Europe and North America initially showed a limited decline, although COVID-19 restrictions were very strict[8]. As countries have expanded their vaccination coverage and employed risk assessments to evaluate the susceptibility and severity of SARS-CoV-2 variants, they have gradually eased the initial restrictions imposed due to COVID-19. Consequently, the diagnosis rate of influenza gradually rebounded, albeit not reaching the levels observed prior to the onset of the COVID-19 pandemic. Then, many countries fully liberalized the epidemic from 2022 to 2023, and the World Health Organization declared on May 5, 2023 that it was no longer a global health emergency[9]. A study from Russia described the high-intensity influenza epidemic in the 2022-2023 season, indicating that influenza activity has fully resumed[10]. The Chinese government decided to adjust the policy and abandoned zero-COVID strategies on December 7, 2022[11]. In recent report in China, Liu et al. identified a massive outbreak of IAV among children in Shanghai after ending the dynamic zero-COVID policy[12]. However, no studies on influenza epidemiology in Hainan Island after the end of dynamic zero-COVID policy have been reported.

Typically, climate factors can affect the spread of viral diseases. While temperate climate regions typically experience influenza epidemics during the winter months, tropical climate regions have infections occurring year-round, leading to more unpredictable outbreaks[13]. Hainan is the only tropical island province in China, located at the southernmost point of China and isolated from the Chinese mainland, experiences a relatively high annual average temperature range of 22.5 °C to 25.6 °C and maintains a consistently humid climate throughout the year. Due to its unique geographical location and climate characteristics, Hainan Island may have even greater challenges regarding viral transmission. Consequently, we carried out a study on the epidemiology and genetic evolution of influenza among children in Hainan Island during the shift phase from dynamic zero-COVID to reopening. Our goal was to gather valuable insights, bolster our readiness for potential influenza outbreaks, and devise more effective preventive measures.

**2 Methods**

From January 2021 to December 2023, we collected 54,974 nasopharyngeal swab samples from pediatric outpatients at the Hainan Women and Children’s Medical Center for IAV testing and 53,151 samples for IBV testing. And 19,687 nasopharyngeal swab samples from pediatric inpatients were collected for IAV and IBV testing. The Hainan Women and Children’s Medical Center is a provincial-level non-profit medical institution, undertaking most of the disease burden for women and children in Hainan province, as well as responsibility of health care and education for women and children. This study was approved by the Ethics Committee of Hainan Medical University. Nasopharyngeal swabs were collected during a patient at presentation or admission and stored at -80°C. Outpatient samples were screened for IVs infection by colloidal gold method. Targeted Next-Generation Sequencing (tNGS) supported by KingMed Diagnostics was used to detect IVs infection from inpatients[14,15].

During this study period, we conducted amplification sequencing on the HA and NA regions of 21 influenza isolates obtained at different stages, resulting in three types: A(H1N1)pdm09, A(H3N2) and B(Victoria).

To ensure a comprehensive phylogenetic analysis, IV isolates were chosen from various influenza prevalent periods,，using primer information from WHO[16]. These isolates were amplified by Nested-PCR. Twenty-one IV-positive samples from inpatients were strategically selected based on their collection times from 2021 to 2023. This selection aimed to effectively capture the genetic diversity and temporal distribution of IV types. Each chosen sample represents a distinct IV type from peak incidence periods, providing crucial insights into the evolutionary trends and subtype variations of IV over the study period. The PCR products were sequenced by Sanger sequencing at the *Tsingke Biotechnology Co, Ltd.* (*Beijing*, China). The sequencing results edited in SeqMan software and identified using the BLAST from National Center for Biotechnology Information (NCBI). Phylogenetic trees were constructed using neighbor-joining and bootstrapping of 1000 replications in MEGA X software. Phylogenetic analyses were conducted on influenza A and B viruses using specific gene segments. For Influenza A virus (IAV), the analysis included A/H1N1 based on a 1131 bp segment of the HA gene and a 906 bp segment of the NA gene, as well as A/H3N2 based on a 754 bp segment of the HA gene and a 926 bp segment of the NA gene. For Influenza B virus (IBV), the analysis included B/Victoria based on a 529 bp segment of the HA gene and a 393 bp segment of the NA gene. Reference strains were selected based on their relevance to the circulating strains in Hainan Island from 2021 to 2023. Additionally, we selected sequences of influenza strains circulating prior to the pandemic from public databases, focusing on strains from various regions in China, including central and southern China, and specifically Hainan. Furthermore, we included strains from Russia, Tajikistan, Thailand, Japan, Cameroon, Togo, Egypt, South Sudan, Chile, the United States, and regions such as Hong Kong. These strains were obtained from the GISAID database, ensuring comprehensive representation of the genetic diversity and evolutionary trends of the influenza viruses[17]. Analysis of the sequences was performed using BioEdit software. The sequencing data were submitted to the NCBI under accession number, from PP732243 to PP732252，PP732220 to PP732225, PP732456 to PP732464, PP736112-PP736121.

Data were analyzed using χ2 test by SPSS (version 25.0) software. Statistical significance was set at p < 0.05. Origin 2021 was used to draw the figures.

**3 Results**

**3.1 Sample information and demographics**

During the study period, a total of 54974 pediatric outpatients underwent IAV testing, and IBV test population numbers of pediatric outpatient were 53151 (Table 1). All the pediatric patients were admitted to Hainan Maternal and Child Health Hospital. The age range of participants for IAV and IBV included individuals aged 0 to 18 years (Table 2). The demographic details of the cases have been summarized in table 1 and 2. In the IAV testing, a median (interquartile, IQ) age is 4.98 years (2.58-7.71), including 32162 males (58.50%) and 22812 females (41.50%). The IBV test population numbers of outpatient children were 53151, consisted of 31,123 males (58.56%) and 22812 females (41.44%), with a median (IQ) age of 5.07 years (2.61-7.77). Nasopharyngeal swab specimens were also collected from hospitalized children with respiratory illness. The median age of the 19687 pediatric inpatient was 2.34 years (1.08-5.06), comprising 12131 males (61.62%) and 22812 females (38.38%). Furthermore, the number of samples collected for IAV antigen detection in 2021, 2022, and 2023 were 7728, 16464, and 30782 cases, accounting for 14.06%, 29.95%, and 55.99% of total outpatient visits, respectively. The number of samples collected for IBV antigen detection were 7812, 16956, and 28383 cases from 2021 to 2023, representing for 14.70%, 31.90%, and 53.40% of the total outpatient visits, respectively. The number of influenza detection samples collected from hospitalized patients were 4721 cases (23.98%), 5227 cases (26.55%), and 9739 cases (49.62%) in 2021, 2022, and 2023, respectively.

**3.2 Epidemiology of influenza among children in Hainan from 2021 to 2023**

During the period of this study, the overall positive rates of IAV among outpatients and inpatients were 28.54% and 5.02%, respectively. The positive rates of IBV among outpatients and inpatients were 5.41% and 1.64%, respectively. Annual incidence rates of IAV and IBV among outpatients and inpatients are represented in Table 1, showing statistically significant differences (p<0.05). There was a noticeable increase in the positive rate of IAV for 2022 and 2023 compared to 2021 (Outpatients: χ2= 3171.13, p=0.00, Inpatients: χ2= 320.18, p=0.00). Additionally, the positive rate of IBV among outpatients in 2023 was higher than in 2022, but lower than in 2021 (χ2= 3681.40, p=0.00). For inpatients, there were no statistically significant differences in the positive rate of IBV between 2023 and 2022, and both rates were lower than in 2021(χ2= 182.71, p=0.00). In the intensive care unit (ICU), IAV positive cases accounted for 0.00% (0/3), 0.11% (33/313) and 0.06% (39/648) from 2021 to 2023 among inpatient pediatric. The number of IBV positive in the ICU were 5, 1, and 7 cases from 2021 to 2023, accounting for 0.03%, 0.02%, and 0.07% of the total IBV positive inpatient infections, respectively.

It illustrates the total number and percentage of IAV and IBV infections per month among outpatients and inpatients from January 2021 to December 2023 (Figure1). Throughout this period, the influenza activities of outpatients and inpatients showed a similar trend, albeit more pronounced in outpatients. Specifically, influenza activity remained subdued until October 2021, when IBV positive rates began to rise, peaking in November, followed by a decline in influenza activity. The IBV infection rate was 39.16% in outpatients and 12.63% in inpatients in November. In 2021, the wave of influenza activity was primarily concentrated in autumn, dominated by IBV infections, and only a small number of IAV cases were detected. The positive rate of IAV increased obviously in May 2022, reached the peak in June, and decreased drastically in July, with an IAV positivity rate of 42.78% among outpatients and 35.04% among inpatients in June. Notably, there was almost no influenza activity in the following six months. In 2022, different from the previous year, influenza activity was obvious in summer with predomination of IAV, while no influenza activity was observed in autumn. Influenza activity did not appear until a high level of IAV was detected in March 2023, reaching its peak in April and declining rapidly in May. In April 2023, there was an IAV infection rate of 41.26% in outpatients and 30.26% in inpatients. During this wave, the entire cycle of influenza activity, from escalation to decline, occurred in spring, two months earlier than the previous year. From June to September 2023, influenza activity remained minimal until a slight increase in IAV and IBV activity in October 2023. Subsequently, high levels of IAV and IBV were detected from November to December 2023, indicating a prolonged influenza wave. Moreover, in December 2023, 31.45% of outpatients and 8.03% of inpatients tested positive for IAV, while 11.82% of outpatients and 4.40% of inpatients tested positive for IBV. The second wave in 2023, the influenza activity commenced in autumn and winter, staring a month later than in 2021. Throughout 2023, two noticeable influenza outbreaks occurred-one in spring predominated by IAV and another in autumn and winter characterized by simultaneous transmission of IAV and IBV, with minimal activity in summer.

Over the three-year study period, the prevalence of influenza seemed to lack a consistent seasonal pattern. As depicted in Figure 2, the outpatient IBV positivity rate reached 29.39% in autumn 2021, while the outpatient IAV positivity rate reached 39.55% in summer 2022 and exceeded 25% in spring, autumn, and winter 2023. To analysis the variation in the activity intensity of IAV and IBV during the study period, we compared the positivity rate changes in the same season over the three years. In 2023, the activity intensity of IAV in spring, autumn, and winter was significantly higher than in the corresponding seasons of 2021 and 2022 (Spring: outpatients: χ2= 388.99, p=0.00, inpatients: χ2=388.50, p=0.00; Autumn: outpatients: χ2= 1192.68, p=0.00, inpatients: χ2=74.25, p=0.00; Winter: outpatients: χ2= 1126.17, p=0.00, inpatients: χ2=146.41, p=0.00), while in summer, it is notably lower than in 2021 and 2022 (Outpatients: χ2= 760.61, p=0.00, Inpatients: χ2=671.77, p=0.00). Regarding influenza B, the activity intensity in autumn 2023 was markedly higher than in 2022 but lower than in 2021 autumn (Outpatients: χ2= 712.57, p=0.00, Inpatients: χ2=175.78, p=0.00).

There was no significant difference in the detection rates of IAV and IBV between genders each year (Table 2, p>0.05). To investigate susceptibility differences among different age groups, the ages were categorized into four groups: 0-1 years (infants), 1-3 years (early childhood), 3-7 years (preschool children), and 7-18 years (school-age children). In 2021, no differences were observed in the detection rates of IAV among hospitalized children in different age groups. However, statistical variances were noted among different age groups in the detection rates of IAV and IBV in other settings (Table 2, p<0.05). In 2021, there were no differences in the detection rates of IAV among outpatient infants, preschool children, and school-age children, while the detection rate of IBV was highest among school-age children in both outpatient and pediatric inpatient, followed by preschool children. In 2022 and 2023, the highest detection rates of IAV and IBV were observed in school-age children among outpatients, followed by preschool children. Before and after the transition from the dynamic zero-COVID policy, two waves of IAV activities occurred, one from May to July 2022 and another from March to May 2023. Comparing the IAV positivity rates during these periods revealed higher rates in infants, early childhood and preschool children among outpatients in 2022 compared to 2023 (0-1: χ2= 25.34, p=0.00; 1-3: χ2= 155.86, p=0.00; χ2= 8.76, p=0.00), however, no significant differences was observed school children (Figure 3). Conversely, the IAV positive rates in preschool children and school children among inpatients were lower in 2022 than in 2023 (3-7: χ2= 6.32, p=0.01, 7-18: χ2=15.60, p=0.00).

**3.3 Phylogenetic characterization of IAV and IBV in Hainan during 2021-2023**

During our study period, we conducted amplification sequencing on the HA and NA regions of 21 influenza isolates obtained at various stages, yielding three types: A(H1N1)pdm09, A(H3N2) and B(Victoria). In sequencing the IAV samples, approximately 1,300 bp of the HA fragment and 1,100 bp of the NA fragment of H1N1 were obtained, covering about 16.33% to 17.66% of the H1N1 genome. For H3N2, approximately 900 bp of the HA fragment and 1,100 bp of the NA fragment were obtained, covering about 13.79% to 14.81% of the H3N2 genome. During the sequencing of the IBV samples, approximately 800 bp of the HA fragment and 400 bp of the NA fragment were obtained, covering approximately 8.28% to 8.39% of the IBV genome. Using reference sequences from databases such as NCBI and GISAID, we constructed six phylogenetic trees based on both HA and NA (Figure 4). Figure 4A and 4B showed the phylogenetic trees of the HA and NA derived from H1N1, with the isolated strains belonging to 6B.1A.5a.2a, one from 2022 and the others from 2023, showing consistent results. Furthermore, the phylogenetic analysis of the HA and NA genes revealed that all five H3N2 isolates in 2022 clustered within the 3C.2a1b.2a.1a clade, while one H3N2 isolate from 2023 fell within the 3C.2a1b.2a.2a.3a.1 clade (Figure 4C and 4D). Notably, the genetic evolution delineated a pattern: the 2022 outbreak was predominantly H3N2, with sequence similarities ranging from 98.10% to 100% based on the HA segment, while the 2023 outbreak was majorly H1N1-09, with sequence similarities for the HA segment ranging from 99.30% to 99.8%. The NA segment analysis echoed these findings, showing sequence similarities between 99.40% and 100%. The phylogenetic analysis based on the HA segment in Figure 4E revealed that all circulating influenza B strains in Hainan during 2021 clustered within a single branch corresponding to the Victoria lineage, which was further subdivided into two subtypes: V1A.3a.1 and V1A.3. Further sequence comparison indicated a high similarity among these strains, with sequence identities ranging from 99.70% to 100% for the HA segment. The analysis from the NA segment corroborated the HA findings, presenting sequence similarities between 98.70% and 100% (Figure 4F). Our data indicates that Hainan experienced three outbreaks of influenza between 2021 and 2023. In 2021, the epidemic was predominantly caused by B(Victoria). In contrast, the epidemics in 2022 and 2023 were primarily driven by influenza A, with A (H3N2) in 2022 and A(H1N1)pdm09 in 2023. Of particular interest is the alternating prevalence of H3N2 and H1N1 genotypes during the influenza A epidemics in 2022 and 2023. As one genotype dominated, the incidence of the other significantly reduced but did not vanish entirely, suggesting a dynamic interplay between these genotypes in the region.

**4 Discussion**

This study investigates the epidemiological characteristics, and genetic diversity of influenza virus circulating in children Hainan Island, both during and following the COVID-19 epidemic. Research consistently demonstrates that children exhibit the highest susceptibility to influenza infection and are at an elevated risk of hospitalization due to their immature immune system[18]. The unique climatic and geographical attributes of Hainan Island, combined with its status as a major tourist destination and a free trade port, significantly influence the transmission dynamics of influenza viruses.

In our findings, Hainan witnessed a significant transition in influenza virus dominance on Hainan Island, shifting from influenza B in the winter of 2021 to influenza A during the summer months of 2022. In 2023, two waves of influenza occurred, initially dominated by influenza A, and subsequently by a concurrent epidemic of influenza A and B. Remarkably, the volume of influenza detections in 2023 exceeded those recorded in the preceding two years, suggesting a resurgence to pre-pandemic influenza activity levels. Influenza typically exhibits seasonal epidemics globally, particularly in temperate regions during the winter; however, in tropical settings such as Hainan, activity can persist year-round[19]. In China, variations in the seasonality and evolutionary dynamics across different regions are common. Prior to the COVID-19 pandemic, northern provinces experienced winter influenza A epidemics peaking in January to February, while southern provinces occurred peak viral activity in spring (April to June). Influenza B activity was more prevalent during colder months throughout most of China[20]. Additionally, provinces at intermediate latitudes exhibited semi-annual epidemic cycles with infection peaks in winter and summer.

The impact of COVID-19 outbreaks and associated NPIs on influenza activity globally declined in 2020, including China, Asia, the US, and Europe, and gradually rebounded in 2021[21, 22]. Interestingly, China witnessed a shift in the distribution of influenza subtypes from A-dominant to B-dominant post-COVID-19 outbreak[23], while our study observed subtypes from B-dominant or A-dominant to co-endemic before and after abandoning the dynamic zero-COVID strategy. This subtype of transformation was consistent with the overall changes in China[24]. The peak of influenza A virus (IAV) activity in the summer of 2022 can be attributed to several factors. Notably, during this period, there was a partial relaxation of some non-pharmaceutical interventions (NPIs), such as localized lockdowns and reduced social distancing measures, which were initially implemented to control the spread of COVID-19[25]. This relaxation potentially facilitated the transmission of influenza viruses. Additionally, the re-opening of schools and resumption of social activities among children likely contributed to increased influenza transmission, as this demographic is highly susceptible to respiratory infections due to high levels of social interaction[26]. In contrast, the peak observed in 2023, post the zero-COVID policy, was influenced by the complete lifting of COVID-19 restrictions. This led to a significant increase in social mixing and travel, thereby accelerating the spread of influenza. Furthermore, the lack of prior exposure to influenza viruses during the stringent COVID-19 control measures might have resulted in reduced population immunity, contributing to a higher susceptibility to influenza infections[27]. Although influenza activity starting to rebound in 2021, our research revealed a period from August 2022 to January 2023 when influenza activity appeared to stalled for over six months without the previous autumn and winter influenza seasons. During the same period, unlike some other countries, for instance, Germany experienced a wave of seasonal influenza A from October 2022 to January 2023[28]. Significantly, August 1, 2022, the Omicron BA.5.1.3 variant was reported in Hainan Province, China firstly. This variant exhibited high transmissibility and susceptibility, causing over 8500 confirmed cases and a period of lockdown across the island[29]. Subsequently, following the discontinuation the dynamic zero-COVID policy on December 7, 2022, China witnessed an upsurge in Omicron variant infections[30]. On the one hand, the implementation of rigorous NPI measures exerted a substantial inhibitory impact on the transmission of influenza virus infections, and children may have been prioritized for COVID-19 vaccination over influenza vaccination[31]. On the other hand, post-COVID-19, the easing of restrictions correlated with a marked increase in influenza activity, notably with an early 2023 outbreak following school re-openings in Hainan. This trend was mirrored in other Chinese regions, such as Shanghai and Henan, indicating a wider pattern of earlier and more severe influenza outbreaks as public health measures were relaxed[12, 32].

Our research indicated that school-age children among outpatients were more susceptible to infection, as supported by the majority of other studies focusing on children[32, 33]. The increased susceptibility of school-age children to influenza, likely due to their high level of social interaction, aligns with observed patterns of higher infection rates in this demographic.

Before the COVID-19 pandemic, global influenza surveillance data showed that both influenza A(H1N1)pdm09, A(H3N2) and B lineages were co-circulating globally following seasonal patterns, with A(H3N2) often being the predominant cause of seasonal epidemics globally[8, 34]. In our country, A(H1N1)pdm09 and influenza B followed an annual cycle, peaking during the winter season, while A(H3N2) exhibited a semi-annual cycle with additional peaks in the summer season in southern China, alongside the annual winter peaks[35, 36]. While 2019 witnessed the cocirculation of A (H1N1) pdm09, A (H3N2), and B (Victoria) strains, 2020 saw a dramatic decline in influenza detections. Interestingly, 2021 experienced an increase, dominated by the B (Victoria) lineage[37]. This limited diversity, in contrast to pre-COVID seasons, contributed to an augmentation in the genetic diversity of the B/Victoria lineage, emphasizing its recurrent emergence since the 1970s[24, 38]. Our study on influenza patterns in Hainan between 2021 and 2023 provides a snapshot of this shift. The dominance of the B/Victoria lineage in 2021 may indicated a localized outbreak resulting from regional spillages or be attributed to strain-specific mutations[39]. Notably, the typical B/Yamagata lineage does not seem to have been globally confirmed since early 2020, suggesting its suppressed or eliminated during the pandemic[8]. In 2021, B/ Victoria dominated in China and a few other countries, while regions like Europe experienced the circulation of A (H3N2) strains[8, 21]. Furthermore, the rising prominence of influenza A in subsequent years possibly mirrors the global interplay between the A and B strains, influenced by changing population immunities and viral adaptability[40]. The oscillating dynamics between H3N2 and H1N1 in 2022 and 2023 underscore the complexity of influenza evolution, shaped by factors such as antigenic drift, temporary cross-immunity, and viral competition[41]. Incidentally, leading up to the 2023 prevalence of influenza A(H1N1) subtype, it has been absent from China for nearly three years. Phylogenetic analysis revealed close relationships among the circulating strains. The selection of 21 IV-positive samples was strategically aimed at capturing a diverse representation of genotypes across various time points. However, we acknowledge that the sample size is relatively limited for making comprehensive conclusions about the overall genetic diversity and evolutionary trends of IVs. Future research with a larger dataset would be beneficial to validate our findings and provide deeper insights into the phylogenetic relationships and variations among IV types. Additional studies are necessary to improve our understanding of the epidemic characteristics and variation trends of influenza viruses.

**5 Conclusion**

In this study, it highlights the profound impact of public health policies on the epidemiological behavior of influenza. The findings presented are integral to deepening our understanding of influenza epidemiology and its complex interactions with other significant infectious diseases, particularly in the context of major global health events like the COVID-19 pandemic. As we move beyond the immediate impacts of COVID-19, it remains imperative to maintain vigilance and preparedness against the possibility of simultaneous circulation of multiple respiratory viruses. Continuous research is essential to accurately characterize the evolving epidemiological trends and variations of influenza viruses, thereby informing effective public health strategies and interventions.

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**Conflict of Interest**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

**Ethics Statement**

This study was approved by the Ethics Committee of the Hainan Women and Children`s Medical Center.

**Data Availability Statement**

All sequences analyzed during this study are available from the NCBI database (GenBank accession No. PP732243-PP732252，PP732220-PP732225, PP732456- PP732464, PP736112-PP736121).

**Author Contributions**

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**Figure legends**

**Figure 1**. Children in Hainan Island, China during 2021-2023. Note: The figure above shows data analysis among pediatric outpatients and below shows data analysis among shows data analysis among pediatric outpatients. January 1, 2021 to December 7, 2022 was the period of during the adhering dynamic zero-COVID policy. August 1, 2022, the Omicron BA.5.1.3 variant was reported in Hainan Province, China firstly. Abbreviations: No., number; IAV, influenza A virus; IBV, influenza B virus; Jan, January; Apr, April; Jul, July; Oct, October.

**Figure 2.** Positive rate of IAV and IBV by season group among children in Hainan Island, China during 2021-2023. (A) IAV and IBV data analysis among pediatric outpatients. (B) IAV and IBV data analysis among pediatric inpatients. Note: The red line shows the positive rate by season in 2021. The blue line shows the positive rate by season in 2022. The yellow line shows the positive rate by season in 2023. Abbreviations: IAV, influenza A virus; IBV, influenza B virus; Spr., spring; Sum., summer; Aut., autumn; Win., winter.

**Figure 3.** Comparison of IAV positive rate by age group among children in Hainan Island between May to July 2022 and March to May 2023. (A) Data analysis among pediatric outpatients. (B) Data analysis among pediatric inpatients. Note: 0-1 years: infants; 1-3 years: early childhood; 3-7 years: preschool children; 7-18 years: school-age children. Vertical bars colored in yellow and green are data in May to July 2022 and March to May 2023, respectively. \* p <0.05, \*\* p <0.001.

**Figure 4.** Phylogenic tree of IAV and IBV among children in Hainan Island, China from 2021 to 2023. (A) Phylogenic analysis of A/H1N1 based on the HA gene. (B) Phylogenic analysis of A/H1N1 based on the NA gene. (C) Phylogenic analysis of A/H3N2 based on the HA gene. (D) Phylogenic analysis of A/H3N2 based on the HA gene. (E) Phylogenic analysis of B/Victoria based on the HA gene. (F) Phylogenic analysis of B/Victoria based on the NA gene. Note: Sequences from this study are colored in red and reference strains are colored in black. The scale bar represents genetic distance; units are the number of base substitutions per site.