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## Detection of RSV in Children $\leq 2$ years from SARS CoV-2 negative samples at National Public Health Laboratory, Nepal

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### Abstract

**Introduction:** Respiratory syncytial viruses also known as human respiratory syncytial viruses (HRSV) are highly transmissible respiratory pathogens & the leading cause of hospitalization due to lower respiratory infection, especially in the pediatric population. This study is intended to determine the RSV positivity in SARS-CoV-2 negative samples obtained from patients  $\leq 2$  years, collected from September to December 2021, when RSV infection is more common.

**Method:** SARS-CoV-2 negative archived nasopharyngeal samples of children  $\leq 2$  years from September to December 2021 at National Public Health Laboratory (NPHL) were tested for RSV by real-time reverse transcriptase Polymerase Chain Reaction (RT-PCR) using CDC RSV Kit. The Pan-RSV primer/probe set used for the universal detection of RSV and duplex RSV subgroup primer/probe set used for the detection and subgroup-specific identification of RSV A and B.

**Result:** A total of 294 SARS-CoV-2 negative samples from children less than 2 y were tested. RSV A was identified in 100(34%) and RSV B was detected in 6(2%) samples. In all, 106(36%) samples were positive for RSV. Here we report RSV positivity rate of 36% in SARS-CoV-2 negative samples which were left undiagnosed during the surge of COVID-19 cases.

**Conclusion:** Our study highlights the need for an accurate and rapid differential or multiplex kits for screening respiratory disease for effective disease management in the pediatric population.

**Keywords:** polymerase chain reaction, respiratory syncytial viruses, SARS-CoV-2

## Introduction

Human respiratory syncytial virus (HRSV) is highly contagious and causes acute respiratory infections worldwide.<sup>1</sup> It is an RNA virus of the family *Paramyxoviridae* and based on antigenic variability classified into two distinct subgroups RSV A and RSV B.<sup>2</sup> Both subtypes often circulate together during epidemic seasons, with subtype A having a substantially higher prevalence than subtype B.<sup>3,4</sup> The virus spreads through the aerosol of the infected people or through direct contact with contaminated fomites as RSV can remain on surfaces for several hours.<sup>5</sup> Typical clinical symptoms vary from mild-common-cold symptoms and can also cause pneumonia, bronchitis, and tracheobronchitis and the consequences may be devastating, particularly in children with a weakened immune system.<sup>6</sup> Most children encounter RSV infection at less than 2 years of age and usually severe requiring hospitalization in under 1 year and death mainly in developing countries.<sup>7,8</sup> Global estimation of RSV as per WHO is 33 million infections with 3 million hospitalizations and 59,600 deaths in hospital and half of the deaths were infants of 6 months annually.<sup>6</sup>

The clinical presentation and mode of transmission of RSV and SARS-CoV-2 are similar, but the management is different.<sup>9</sup> During the pandemic, the most attention remains focused on COVID-19 testing while RSV infection remains to be uncovered. In this study, COVID-19 negative samples of children of  $\leq 2$  years of age were examined for RSV positivity. The findings of this study help to identify the presence of RSV and raise awareness for optimum diagnosis and management.

## Method

This is a descriptive cross-sectional study conducted at the National Influenza Center (NIC), National Public Health Laboratory involving a review of secondary data from electronic database records. NIC at NPHL is the recognized center for coordinating integrated

influenza and SARS-CoV-2 surveillance. This was hospital-based sentinel surveillance including 16 hospitals selected based on geographical region and population density with the provision of sending representative respiratory samples on a weekly basis. Likewise, this included other collaborating laboratories to share test results to upload in the RESPIMART platform.

For the study, we included all acceptable samples i.e., volume more than 200  $\mu$ l and of good quality COVID-19 negative nasopharyngeal archived samples of children  $\leq 2$  years for RSV testing by using Centre for Disease Control (CDC) RSV Real Time Polymerase Chain Reaction (PCR) Kit. The samples received from September 2021 to December 2021 were included in the study.

COVID-19 laboratory results from September to December 2021 were obtained from the dedicated Software (Dolphin). The file was exported to MS EXCEL and the COVID-19 negative results of children  $\leq 2$  years were sorted and saved in a spreadsheet. As this study involved analyzing retrospective data from routine records, the need for informed consent was waived; data confidentiality was maintained throughout the study period by maintaining the anonymity of subjects from whom samples were obtained.

The ethical approval for the study was obtained from the National Health Research Council (169-2022).

From September 2021 to December 2021, a total of 483 samples of children  $\leq 2$  years were received for COVID-19 testing at NPHL. Of 483 archived samples, 17 were COVID-19 positive and 172 were less than 200  $\mu$ l which was not enough for testing and hence excluded from the study. The remaining 294 samples were considered eligible and included in RSV testing.

The samples were processed using the RSV PCR CDC kit (catalog: RSV\_RUO-01) protocol suggested by the CDC.<sup>10</sup> The Pan RSV primer/probe set was used for the universal detection of RSV and duplex RSV subgroup

primer/probe set was used for the detection and subgroup-specific identification of RSV A and B. In the first round, the PCR reaction mixture consisted of 5 µl nuclease-free water, 1.5 µl of combined primer/probe mix, 1 µl of 25X RT PCR enzyme mix, 12.5 µl of 2X RT PCR buffer, and 5µl of extracted RNA. In the first round, the cycling conditions for the PCR reaction were set up as reverse transcription at 45°C for 10min, RT inactivation at 95°C for 10 min, followed by 45 cycles of denaturation and extension at 95°C for 15 s, 55°C for 60 s respectively in the ABI 7500. The reporter dye (FAM for RSV Assay), quencher dye none, and (RP-FAM) were used and processed the test following the result interpretation. Those samples which reported as positive for RSV further subgroup-specific identification of RSV A & B using RSV A/B combined primer-probe set instead of RSV combined primer-probe following the same calculation, process, and cycling condition as mentioned above for the RSV detection was carried. The detector dye FAM for RSV A and Texas Red for RSV B were used. Positive and negative controls were included in each run. The CT value of less than

40 was reported as positive. The result was compiled in the Excel sheet and analysis was done.

## Result

Out of 294 processed COVID-19 negative samples, 106(36%) samples were reported positive for RSV by the real-time RT-PCR. Maximum number of positive cases, 57(68%), were reported in the month of September, Figure 1. Overall declining trend was seen with the least positive cases (nil) in the month of December.

Subtype-wise, RSV A was the predominant subtype 100(94.3%) during the study period. Only 6(5.6%) RSV B was detected and only in the samples collected during the month of September. Gender-wise, the male population was seen to be more affected, with 184(62.5%) positivity. The most affected age group was between one to two years, where 167(58%) reported positive.

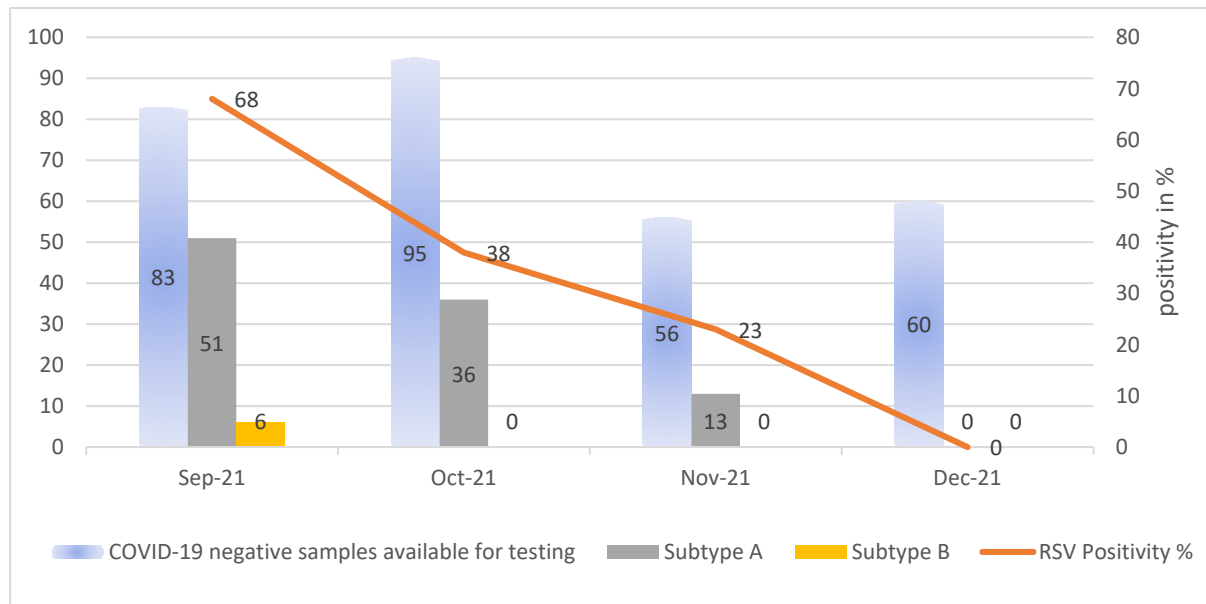


Figure 1. RSV Positivity in COVID-19 negative samples from Sep 2021-Dec 2021 of children ≤2 y

## Discussion

All novel respiratory viruses have varying but significant impacts on human health, however, 12-39% of etiological agents causing

respiratory infections remain unidentified.<sup>11</sup> Globally, RSV is one of the major respiratory viruses associated with a significant burden of respiratory illness, particularly in young children, and a common cause of repeated

infection but data are limited from South Asian countries.<sup>12,13</sup> In India, the rates of RSV detection in studies on younger children (ages 0-5) range from 2.1% to 62.4%.<sup>14</sup> Similarly, according to a meta-analysis study from China, 16.0% of Chinese children with acute lower respiratory tract infection (ALRTI) also had RSV infection, which commonly affects children under 3 years old, especially those under 6 months.<sup>15</sup> A study done in Japan showed RSV hospitalizations occurred at a rate of 23.2 per 1,000 person-years in children under the age of two, rising to 35.4 per 1,000 in infants under the age of six months.<sup>16</sup> Similar to this, a study conducted in Sri Lanka found that 28% of children under the age of 5 had RSV-associated ARTI.<sup>17</sup>

In Nepal, there is a dearth of information available to the general people regarding the epidemiology of RSV and its clinical features in children. Data gaps regarding the burden of RSV infection point to the need for additional studies to better understand the burden of this illness. However, this study with a limited number of samples received over a short period of time has shown 36% RSV positivity in COVID-19-negative samples. The positivity was as high as 68% in September and declined thereafter, providing a hint about this seasonal trend. A study conducted across 27 countries also found that RSV wave started between March to June in southern hemisphere countries and between September to December in northern hemisphere countries.<sup>18</sup> RSV incidence appears to peak from July to November.<sup>19</sup>

RSV divides into two major antigenic subgroups, RSV-A and RSV-B, based on the glycoprotein antigen. The subgroup is further divided into 20 RSV A genotypes and 39 RSV B genotypes to date based on gene sequence.<sup>2,20</sup> Moderate bronchiolitis, fever for >4 days, dyspnea, and tachypnea were significantly associated with RSV-A infection. Mild to moderate bronchiolitis, fever for >4 days, headache, severe dehydration, and cough were significantly associated with RSV-B infection.<sup>17</sup> There was a significant difference in increased disease severity caused by RSV- A

and likely to require intensive care treatment compared with children with RSV-B consistent with reports in different studies.<sup>21,22</sup>

Both types may circulate simultaneously one subtype becomes superior to another depending upon the epidemic region and climate.<sup>5</sup> Our study found subtype A to be more predominant than subtype B (94.3% vs 5.6%) which is similar to other studies.<sup>23,19</sup> Some studies have also observed RSV A to be predominating in some year and RSV B in other years.<sup>24</sup> Studies from India show RSV type A was prevalent in the year 2016 and 2017 and RSV type B was predominant in 2018.<sup>20</sup> Similarly, a study from India also showed the presence of both RSV subtypes, but the predominated RSV B.<sup>25</sup> There was a domination of different subtypes in different seasons in each country.<sup>26</sup> This comparison cannot be made from our study as we have not studied samples from a different year.

Respiratory viral infection has overlapping symptoms, making it challenging to diagnose a specific respiratory viral infection without a solid laboratory diagnosis.<sup>27</sup> Various methods for laboratory diagnosis such as viral culture, immunofluorescence test, Nucleic Acid Amplification test (NAAT), and point of care testing have been developed for the appropriate treatment. Conventional diagnostic methods such as viral cell culture require advanced cell culture laboratory and time consuming whereas immunofluorescence test has limited sensitivity.<sup>28,29</sup> Nowadays highly sensitive and NAAT using real-time PCR assays are widely used diagnostic techniques, which can be carried out using a single set of primers or multiplex formats following the design of probes and primers and laboratory optimization.<sup>27,28</sup>

In our study, we used molecular RT PCR uniplex test for the identification of RSV. Instead of uniplex tests various conjugation assays like multiplex assay or Xpert Xpress Flu/RSV which provide fast, reliable as well as detect multiple pathogens at a time can be used.<sup>30,31</sup> Other than RT PCR tests, rapid identification tests are also available that provide results within a

short time that help to improve patient outcomes; however, it is still in debate.<sup>32</sup> The effective diagnosis of viral etiology helps in providing correct treatment and antiviral therapy that helps in patient management.

Introducing RSV testing as a part of integrated surveillance in respiratory syndromes can be well considered to find out burden of disease in the targeted population. This surveillance data will be helpful for the health workers and policy makers for timely planning, leading to early responses and preparedness in due course.

The limitation of our study is that the study period and the number of collected samples were not enough to show more comprehensive and precise epidemiological features. We also lack data on RSV trends before COVID-19 to compare the impact of COVID-19 on the pattern of RSV infection. Many of the studies show the implementation of non-pharmaceutical intervention measures, such as masks, physical distance, and hand hygiene significantly impacted the transmission of RSV.<sup>26,33</sup> This study also does not reflect the coinfection, subtype prevalence, and overall condition of the patient presentation, thereby this will be a promising topic in our future study with a large sample size and duration. The RSV poses a threat to infants and young children as no vaccine is currently available.<sup>20</sup> Thus, it is highly recommended to introduce RSV testing, both as a routine diagnostic test and as part of a surveillance program by increasing and strengthening the sentinel site for patient management and disease transmission prevention at an apparent time.

Besides, in view of the limitation of this study with the context of time span and sample volume, a study of longer duration, at least a year, comprising of adequate number of samples is warranted, which can give a more solid perspective on seasonality and trend of the RSV infection in children in Nepal.

## Conclusion

Though the study was conducted on a limited number of samples collected primarily for COVID-19 testing, this study has clearly shown that RSV is prevalent in the country and can be considered as a common cause of respiratory infection in children below 2 years. This finding implicates consideration of introducing RSV testing as one of the differential diagnosis for children, particularly below 2 years of age, presenting with acute respiratory syndrome in hospitals.

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## Conflict of Interest

None

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None

## Author Contribution

Concept, design and planning: RJ, PJ, LS; Literature Review: RJ, PJ, LS, LBC; Sample Processing / Analysis: PJ, LBC; Draft Manuscript: PJ, LS; Revision of draft: RJ, PJ, LS; Final Manuscript: RJ, PJ, LS.

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