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Prevalence, pattern of distribution and characterization of respiratory syncytial virus associated acute respiratory tract infections in hospitalized children less than 5 years in a general hospital in Sri Lanka from 2016–2018

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ABSTRACT

Respiratory Syncytial Virus (RSV) is one of the most common respiratory viruses causing acute respiratory tract infections (ARTI) in children. Detailed data on RSV infections including the RSV types circulating in Sri Lanka are not available. This study aimed to determine the prevalence, patterns and characterization of RSV associated ARTI in hospitalized children less than 5 years in a general hospital in Sri Lanka. We tested 500 nasopharyngeal aspirate (NPA) samples collected from children with suspected viral ARTI from May 2016 to July 2018 from Kegalle General Hospital, Sri Lanka for RSV using antigen detection by an immunofluorescence assay (IFA). RSV positive samples were further characterized using the real time RT-PCR. RSV was the predominant virus associated with ARTI with a prevalence of 28% (140/500) in the study sample. RSV in was also detected in more co-infections with other respiratory viruses. RSV was detected throughout the year with peak periods from June to August 2016, March to July 2017 and May to July 2018. Of the 140 RSV positive children tested, 72.14% had RSV-B, while 27.86% had RSV-A infection. Both RSV subtypes were detected throughout the study period with overlapping patterns. A few co-infections between RSV-A and RSV-B were detected during the co-circulation. RSV was the most prevalent virus and RSV-B was the predominant subgroup associated with ARTI in the children <5 years in Sri Lanka from May 2016 to July 2018. RSV was detected throughout the study period with peaks in certain months in the study area.

1. Introduction

Respiratory syncytial virus (RSV) recently renamed as human orthopneumovirus has been identified as a leading cause of acute respiratory tract infection (ARTI) in infants and children globally [1,2]. High rates of hospital admissions of children and infants due to RSV associated ARTI cause a substantial burden to the healthcare systems around the world. According to the estimates made in a global burden study, in children <5 years, 33.1 million episodes of RSV associated ARTI have been reported with 3.2 million hospital admissions and 59600 in-hospital deaths [2]. It has been estimated that more than 93% of all RSV associated ARTI episodes and 99% of the RSV associated ARTI mortalities in children occur in developing nations [3].

RSV infection is seasonal in most countries and RSV activity varies according to the geography and the altitude of a region. In temperate and Mediterranean climates, RSV activity peaks in cold seasons during the fall or winter. In temperate and sub-tropical regions, RSV activity positively correlates with high relative humidity and low temperature [4]. In tropical climates, RSV associated ARTI outbreaks occur mostly in wet seasons following the seasonal rainfall and RSV transmission reach its peak after seasonal rainfalls [5]. Countries located close to the equator experiencing high perennial rainfall and large islands like Singapore, Colombia and Hawaii show a distinct pattern of RSV activity, occurring throughout the year or one half of the year. Neither rainfall nor temperature act as the determinant for RSV outbreaks in these regions and thus RSV outbreaks show periodic emergence in these regions and the reason for such periodic emergence is not clear [6–8].

Abbreviations: RSV, respiratory syncytial virus; ARTI, acute respiratory tract infection; NPA, nasopharyngeal aspirate; Inf V, influenza virus; HPIV, Human Parainfluenza virus.

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RSV is classified into two antigenic groups, A and B based on the primary differences identified between the two groups on the major attachment protein (G) gene [9]. The RSV-A and -B are further classified into 14 RSV-A genotypes and 26 RSV-B genotypes [10–18]. The whole-genome evolutionary analyses show that RSV-A and RSV-B display similar evolutionary rates and degrees of conservation [19]. The molecular epidemiology of RSV is very complex and RSV genotypes co-circulate during a single epidemic resulting temporal and geographic clustering of a particular genotype [20].

Continuous surveillance of RSV is vital to identify the disease burden, patterns of outbreaks and circulating RSV subtypes. Characterization of RSV provides a better understanding of the circulating virus and thus helps in the development of anti-RSV drugs, vaccines and pro-phylactic approaches. There are no detailed studies done on RSV associated childhood ARTI in Sri Lanka with a larger sample size over a longer period. Hence, the current study was conducted over a 26 month period in a sample of 500 hospitalized children to identify the circulating RSV subtypes and the pattern of distribution of RSV associated childhood ARTI in Sri Lanka.

2. Materials and methods

2.1. Study design and study population

This is a prospective cohort study and the ethical approval (Permit No: 2016/EC/91) was obtained from the Ethical Review Committee of the Faculty of Medicine, University of Peradeniya, Sri Lanka. A total of 500 nasopharyngeal aspirate (NPA) samples were collected from children under 5 years hospitalized at the Teaching Hospital, Kegalle, Sri Lanka, between May 2016 and July 2018. The samples were collected from hospitalized children with suspected viral ARTI using the WHO's classification and case definitions of ARTI [21,22].

2.2. Sample collection and laboratory processing

NPA samples were collected from hospitalized children with a history of ARTI of <4 days, recurrent RTI and hereditary or anatomical anomalies in cardiovascular and respiratory systems with ARTI. Children hospitalized with ARTI between the ages of < 1 month and > 5 years with suspected or established bacterial RTI and children not consenting for collecting NPA were excluded. A written informed consent was obtained from parents or guardians of children enrolled in the study.

The NPA samples were collected using a recommended mucus extractor (Pacific Hospital Supply Co. Ltd, USA) and diluted in phosphate buffered saline (PBS) then processed by multiple centrifugation and vortexing steps until the cell sediment was formed. Then the specimen for direct testing was prepared by adding 1000 µl of PBS to re-suspend the cell pellet. The processed NPA samples were stored at 4˚C for a maximum of 24 h for antigen detection and then the samples were frozen at -80˚C until processed for viral RNA extraction and further testing.

2.3. Antigen detection by immunofluorescence assay

Antigen detection for RSV in NPA was carried out using an immunofluorescence assay (IFA). D3 Ultra Respiratory Virus Screening and ID Kit (Diagnostic Hybrids, USA – Catalog No: 01-010000.v2) was used to detect seven viruses: RSV, influenza-A (Inf-A), Inf-B, adenovirus, human para-influenza -1 (HPIV-1), HPIV-2, and HPIV-3. Fluorescence microscopy (LeitzDiaplan Fluorescent Microscope, Germany and Zeiss Axio-cam Fluorescent Microscope, Germany) was used to detect the cells expressing specific antigens for the seven viruses (Supplementary Figs. 1 & 2).

2.4. Viral RNA extraction and real-time reverse transcription polymerase chain reaction

Nucleic acid extraction from RSV positive samples by IFA was conducted using QIAamp Viral RNA Mini Kit (Qiagen, Germany – Catalog No: 52906). Extracted RNA were tested by a real-time reverse transcription polymerase chain reaction (rtRT-PCR) for RSV typing (The RealStar® RSV RT-PCR Kit 3.0 Altona Diagnostics, Germany – Catalog No: 193013) using the Rotor-Gene 6000 real time PCR machine and Rotor-Gene Q Series Software 2.3.1 -Build 49 software (Corbett Life Science, Australia).

3. Results

A total of 237 (47.4%) children were positive for the respiratory viruses including RSV by IFA screening. Overall, 140 of the 237 respiratory viruses positive children (59.07%) were positive for RSV; 22 (9.28%) children were positive for Inf-A; 19 (8.01%) children were positive for Inf-B; 3 (1.26%) children were positive for HPIV-1; 6 (2.53%) children were positive for HPIV-2 and 19 (8.01%) children were positive for HPIV-3 (Fig. 1). A total of 28 (11.81%) children were co-infected and of that, 27 children had dual co-infections and 1 child had a triple co-infection. In the current study, RSV was the most predominant respiratory virus detected with the highest prevalence compared to other viruses and majority of the co-infections were associated with RSV (Fig. 2). The median age and male to female ratio of RSV infected children in the current study sample was 10.19 months and 1.7: 1, respectively.

Based on the findings of the current study, RSV was prevalent throughout the year with peaks in certain months. Major RSV peaks were observed from June to August 2016, March to July 2017 and May to July in 2018. Minor RSV peaks were observed from October to November 2016 and September 2017. RSV-B was the most dominant subgroup of RSV circulated in the study sample in 2016 and 2017. RSV-A gradually increased during the study period and reached its highest prevalence during its last peak in May 2018. Co-infections between RSV-A and RSV-B were detected during the periods of co-circulation of these subtypes (Fig. 3).

RSV positivity increased in peak periods every year. RSV-B was observed throughout the study period with peak incidence from June to August in 2016, March to June in 2017 and April to June in 2018. RSV-A was also detected throughout the year with a lesser incidence than RSV-B with peaks from March to July in 2017 and April to June in 2018. RSV-A incidence during the 2018 outbreak (April to June 2018) was significantly higher compared to that noted in 2016 and in 2017 outbreaks. In 2018, RSV-A incidence was more or less similar to the RSV-B incidence reported in that year (Fig. 4). Co-infections between RSV-A and RSV-B were detected during the periods of co-circulation of both types.

4. Discussion

The present findings provide details on RSV disease burden and circulating RSV sub types in children in the Kegalle area of Sri Lanka from May 2016 to July 2018 and this is the first report on the RSV subtypes circulating virus in Sri Lanka. Prevalence of RSV/RSV sub types, co-infections of RSV with other viruses, distribution and seasonality of RSV are in agreement with those reported elsewhere [5,23,24]. RSV was the predominant respiratory virus associated with ARTI in the study population and RSV had more co-infections with other respiratory viruses. RSV is responsible for 27 to 96% of hospitalizations of children with viral ARTI in temperate, tropical and developing countries [5]. Moreover, severe RSV associated ARTI is a major cause of mortality in children and 99% of those deaths occur in developing countries [25]. The importance of RSV is underestimated in developing countries due to the lack of routine viral diagnostics linked with limited resources. Thus children with

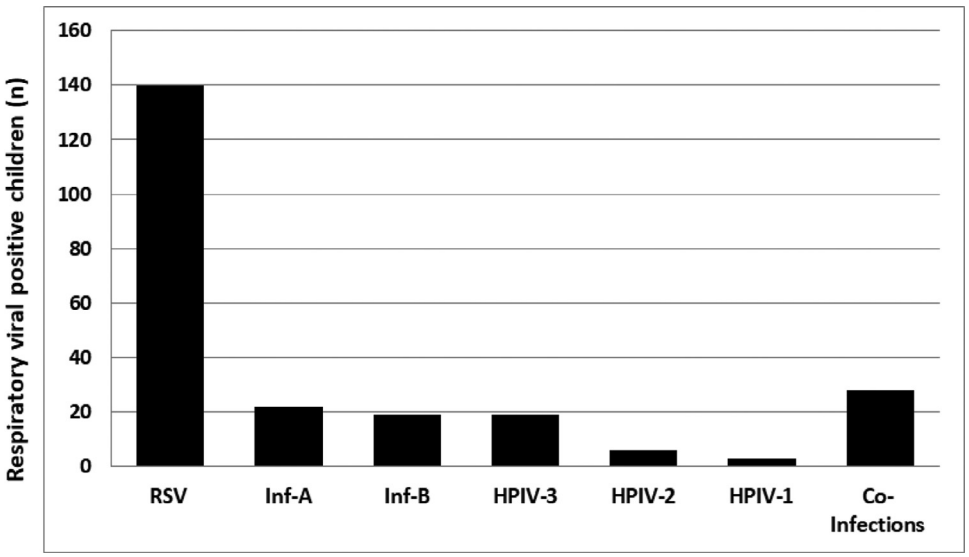

Fig. 1. Prevalence of respiratory viral mono- and co-infections in the study sample. RSV was the predominant respiratory virus detected in the study population with 140 mono-infections out of the 500 children.

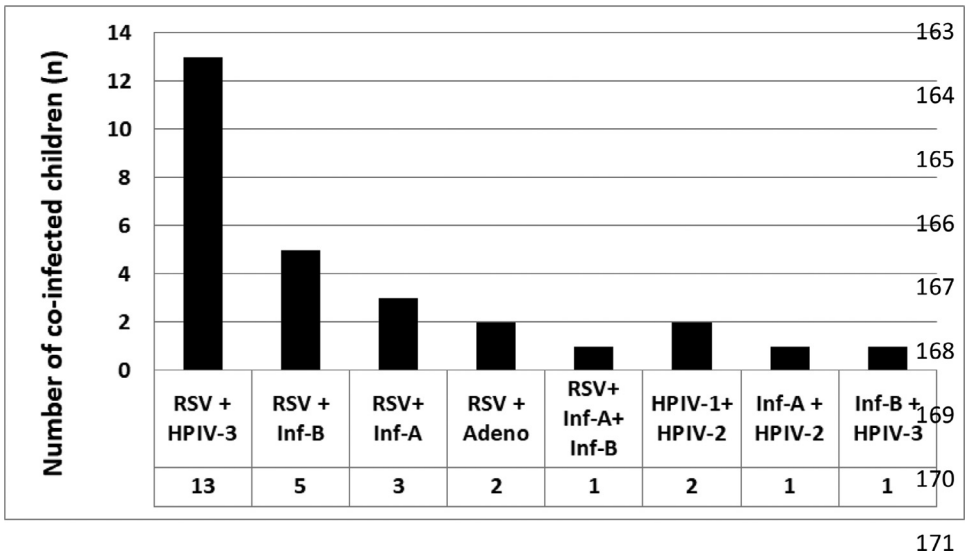

Fig. 2. Prevalence of respiratory viral co-infections in the study sample. A total of 24 out of the 28 co-infections were associated with RSV (85.71%).

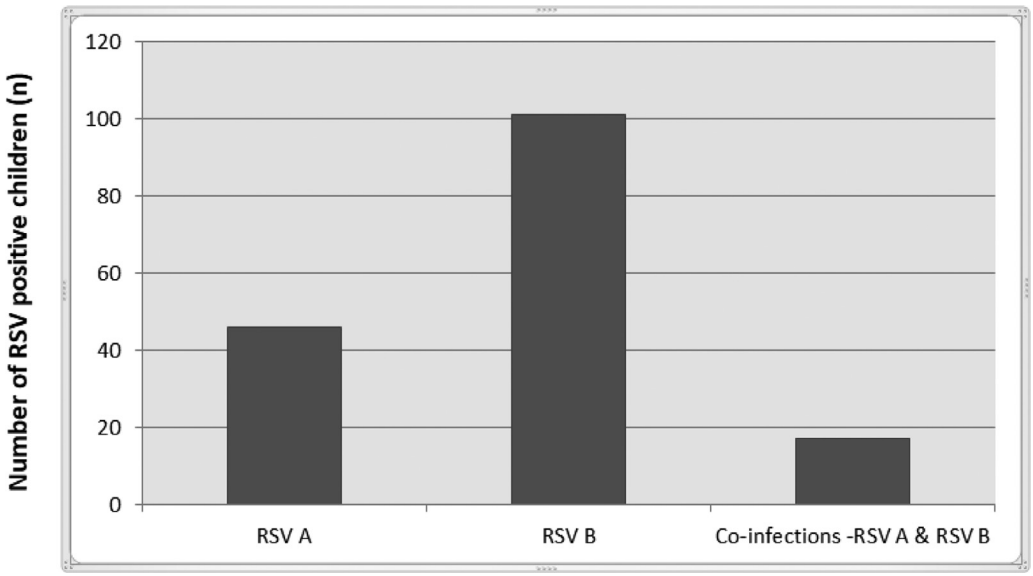

Fig. 3. Distribution of RSV subtypes showing the predominance of RSV-B in the study sample. Of the 164 children positive for RSV by IFA, 46 were infected with RSV-A, 101 were infected with RSV-B and 17 were co-infected with RSV-A and RSV-B real time RT-PCR.

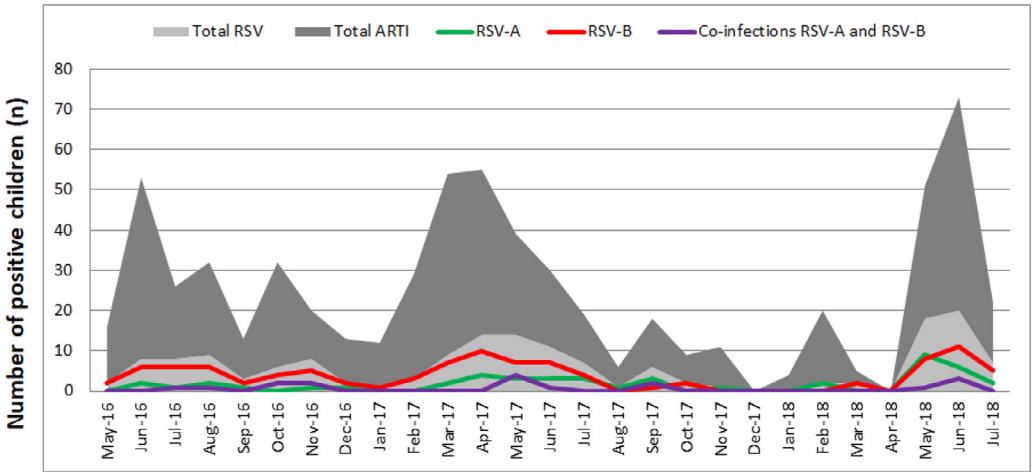

Fig. 4. Monthly distribution of RSV infected children in the study sample for a period of 26 months from May 2016 - July 2018. RSV was distributed throughout the study period with peaks occuring in certain months. Major RSV peaks were observed from June to August 2016, March to July 2017 and May to July 2018. Minor RSV peaks were observed from October to November in 2016 and September in 2017.

ARTI are usually treated with antibiotics without viral diagnostic evidence [5]. Lack of diagnosis and management would further aid the transmission of RSV contributing to mortality in children in developing countries. RSV co-infection with other respiratory viruses are associated with the longer hospital stay, which also facilitates hospital acquired viral infections [26].

In the current study, RSV-B was the dominant subgroup circulated in the study area from May 2016 to July 2018. Both RSV subtypes were observed throughout the study period with peak incidence in some months. The overlapping periods of RSV-A and RSBV-B resulted with co-infections between the two subtypes. The dominant circulation of one RSV subtype and co-infections between the two RSV subtypes during the times of co-circulation are indistinguishable from previous studies [24,27]. According to Bose *et al* (2015), multiple genotypes of RSV co-circulate in the world with some predominant subtypes circulating within defined regions over sustained periods or fluctuations happening in different years [27]. Likewise, many studies have observed co-infections of RSV with other respiratory viruses as well as co-infections between RSV-A and RSV-B genotypes [24,28,29].

RSV was prevalent throughout the year with major peaks occurring in June to August in 2016, March to July in 2017 and May to July in 2018. Similar RSV peaks have been observed in other studies conducted in Sri Lanka, which suggests the influence of coinciding monsoonal rains on RSV associated ARTI [30–32]. RSV infection is seasonal in most countries and RSV seasonality varies considerably between regions [5]. Moreover, high RSV associated mortality rate in children outside the RSV season in tropical and subtropical countries suggests the year-round RSV activity in those regions [25].

5. Conclusion

In the current study, RSV was the most predominant viral cause of childhood ARTI and hospitalization. RSV-B was the most common subtype circulated in the study sample. RSV co-infections between major RSV subtypes and other viruses (Inf-A, Inf-B, HPIV-1, HPIV-2 and HPIV-3) were also detected. RSV was prevalent throughout the study duration with peak periods in certain months. Both RSV subtypes RSV-A and RSV-B were detected throughout the study period. This study provides detailed information on prevalence, patterns of distribution and circulating subtypes of RSV in children less than 5 years of age and these findings will help implement common respiratory infection control methods at peak prevalence periods against RSV infection in Sri Lanka.

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Declaration of Competing Interest

The authors declare no conflict of interest.

CRediT authorship contribution statement

Maduja VM Divarathna: Data curation, Funding acquisition, Writing – original draft. **Rukshan AM Rafeek:** Data curation, Writing – review & editing. **Sampath Jayaweera:** Data curation, Writing – review & editing. **Adrian J Morel:** Conceptualization, Data curation, Project administration. **Faseeha Noordeen:** Conceptualization, Project administration, Writing – review & editing.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcvp.2022.100107.

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