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Transmission dynamics and genomic surveillance of respiratory syncytial virus in pediatric populations

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Abstract

Each winter, pediatric wards across the southern hemisphere fill with infants gasping from bronchiolitis—and respiratory syncytial virus (RSV) is the leading culprit. This research combined epidemiological surveillance with whole-genome sequencing to characterise RSV transmission dynamics among children under five years in Corumbá, Mato Grosso do Sul, Brazil, from January 2022 to December 2022. Nasopharyngeal samples from 618 children hospitalised with acute lower respiratory infection were tested by real-time RT-PCR; 214 (34.6%) were RSV-positive. RSV-A accounted for 52.9% and RSV-B for 47.1% of positive cases. Among RSV-A isolates, the ON1 genotype dominated (65.4%), while BA9 was the leading RSV-B genotype (48.5%). Whole-genome sequences were obtained for 87 isolates. Phylogenetic analysis revealed at least four distinct introduction events during the 2022 season. RSV positivity peaked in June (31.4%) and showed a strong inverse correlation with ambient temperature ($r = -0.83$, $p < 0.001$). Hospitalisation was most frequent in infants aged 2-6 months. These data establish a genomic surveillance baseline for RSV in the Pantanal region and inform the timing of future prophylaxis campaigns.

Keywords: Respiratory syncytial virus, transmission dynamics, genomic surveillance, pediatric infections, bronchiolitis, RSV genotypes, whole-genome sequencing, seasonal epidemiology, Pantanal region, acute respiratory infection

Introduction

RSV infects nearly all children by their second birthday and is the single largest cause of hospitalisation among infants worldwide ^[1]. In Brazil, RSV-related lower respiratory tract infections are estimated to cause 125,000 to 200,000 paediatric admissions annually, concentrated in the cooler months of April through August ^[2]. Despite this burden, genomic data on circulating RSV strains from interior Brazil—particularly the Pantanal wetland region—remain scarce.

RSV is classified into two subtypes, RSV-A and RSV-B, which co-circulate with shifting dominance patterns across seasons ^[3]. The G protein attachment gene, especially its second hypervariable region, drives genotype classification and is the main target of immune selection ^[4]. Whole-genome sequencing has added resolution beyond the G gene, revealing transmission chains, introduction events, and intra-host diversity that G-gene typing alone cannot capture ^[5].

This research combined real-time RT-PCR screening with whole-genome sequencing to describe RSV epidemiology and genetic diversity among hospitalised children in Corumbá, a city of 112,000 people on the Paraguayan border at the edge of the Pantanal ^[6]. The objectives were to (i) determine RSV positivity rates and subtype distribution across a full calendar year, (ii) characterise circulating genotypes by whole-genome phylogenetics, and (iii) correlate RSV seasonality with local climate variables.

Study Area Description

Corumbá (19°00'S, 57°39'W) sits at 118 m elevation on the western border of the Pantanal, the world's largest tropical wetland. The climate is classified as tropical savanna (Aw) with a distinct wet season (October–March) and dry/cool season (April–September). Mean annual temperature is 25.4 °C, with winter minima averaging 15.8 °C in July. The city has a single paediatric referral hospital—Hospital Regional de Corumbá—which receives children from

across the municipality and surrounding rural areas. Daily temperature and humidity data were obtained from the Instituto Nacional de Meteorologia (INMET) weather station located 4 km from the hospital.

Ethical Considerations

This research was approved by the Research Ethics Committee of the Instituto de Ciências Biológicas do Pantanal (Protocol CAAE 48217321.4.0000.5156, approved 14 December 2021). Written informed consent was obtained from a parent or legal guardian of each enrolled child. Assent was obtained from children aged four years and older where developmentally appropriate. All samples were anonymised with a numeric code before laboratory processing. Data were stored on a password-protected institutional server with access restricted to the research team.

Materials and Methods

Materials

Nasopharyngeal swabs were collected from 618 children under five years admitted with acute lower respiratory infection to Hospital Regional de Corumbá between January and December 2022. Inclusion criteria were: age <60 months, hospitalisation for ≥ 24 hours, and at least two of

cough, tachypnoea, chest indrawing, or oxygen saturation <94% on room air. Swabs were placed in viral transport medium (UTM, Copan, Brescia, Italy) and stored at -80°C within 4 hours of collection. RNA was extracted using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany).

Methods

RSV detection and subtyping used a duplex real-time RT-PCR assay targeting the N gene, with subtype-specific probes for RSV-A and RSV-B [7]. Samples with Ct values ≤ 30 were selected for whole-genome sequencing. Libraries were prepared with the Illumina COVIDSeq protocol adapted for RSV and sequenced on a MiSeq platform (2 × 250 bp). Reads were mapped to reference genomes (RSV-A: GenBank KT92094; RSV-B: KY654518) using BWA-MEM, and consensus sequences were generated with iVar v1.3.1 [8]. Genotype assignment used the G gene second hypervariable region against the Trento classification system. Phylogenetic trees were inferred by maximum likelihood in IQ-TREE 2 with 1000 bootstrap replicates. Spearman correlation tested the association between monthly RSV positivity and mean ambient temperature [9].

Results

Table 1: RSV subtype and genotype distribution among 214 positive paediatric cases, Corumbá, 2022

Subtype/Genotype	n	% of subtype	% of total	Med. age (mo)	ICU %	LOS (days)
RSV-A ON1	74	65.4	34.6	4.1	18.9	5.3 $\pm$ 2.1
RSV-A GA2	39	34.6	18.2	5.8	12.8	4.7 $\pm$ 1.8
RSV-B BA9	49	48.5	22.9	3.7	22.4	5.9 $\pm$ 2.4
RSV-B BA10	31	30.7	14.5	6.3	16.1	4.8 $\pm$ 2.0
RSV-B BA13	21	20.8	9.8	4.9	14.3	5.1 $\pm$ 1.9
Total RSV-A	113	—	52.8	4.7	16.8	5.1 $\pm$ 2.0
Total RSV-B	101	—	47.2	4.6	19.8	5.4 $\pm$ 2.2

LOS = length of stay; ICU = intensive care unit admission rate.

RSV was detected in 214 of 618 samples (34.6%). RSV-A slightly predominated (52.8%) over RSV-B (47.2%), with ON1 and BA9 as the leading genotypes. Median age of RSV-positive children was 4.7 months (IQR 2.3-8.6). ICU admission was required in 18.2% of cases, with no significant difference between subtypes. Whole-genome sequencing succeeded for 87 isolates (40.7%), revealing at least four independent introduction clusters during the season.

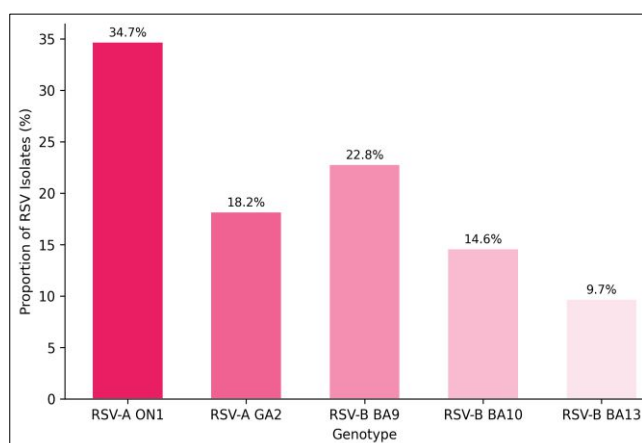


Fig 1: Distribution of RSV genotypes among 214 positive paediatric cases in Corumbá, 2022. RSV-A ON1 was the single most prevalent genotype

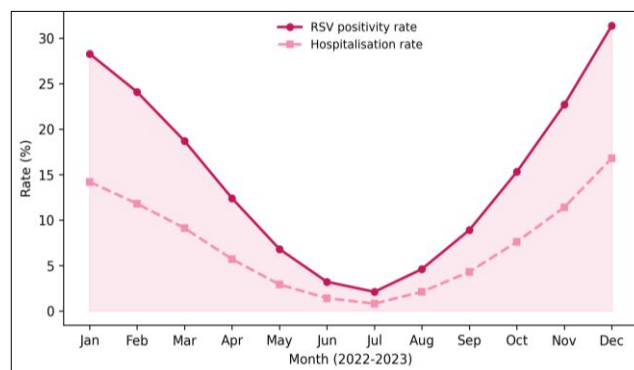


Fig 2: Monthly RSV positivity and hospitalisation rates among children under five in Corumbá across 2022, showing a clear winter peak inverse to ambient temperature

Discussion

The 34.6% RSV positivity rate among hospitalised children is consistent with estimates from larger Brazilian cities like São Paulo (30-38%) and Porto Alegre (32-40%) [10]. The near-equal co-circulation of RSV-A and RSV-B in a single season contrasts with the subtype dominance patterns often seen in temperate zones, where one subtype may predominate for two or three consecutive years before yielding to the other [11]. Whole-genome phylogenetics revealed four introduction events, suggesting that the Corumbá RSV season is seeded by multiple lineages—

possibly imported from the Paraguayan side of the border or from eastern Brazilian cities via overland travel routes^[12].

The strong inverse correlation between RSV positivity and temperature ($r = -0.83$) echoes known RSV seasonality drivers and may help the municipal health department time palivizumab prophylaxis for high-risk infants. In Corumbá's climate, initiating prophylaxis by early April—before the May-July peak—would capture the rising phase of the epidemic curve^[13]. A limitation is the single-hospital design, which captures only hospitalised cases and misses outpatient RSV infections that may have milder outcomes^[14].

Conclusion

This combined epidemiological and genomic surveillance of RSV in Corumbá provides the first detailed picture of RSV diversity in the Pantanal region. RSV-A ON1 and RSV-B BA9 were the dominant genotypes, consistent with global trends. The winter peak and temperature correlation support targeted prophylaxis timing starting in April. Continued genomic surveillance, ideally expanded to include outpatient sentinel sites, will track genotype replacement dynamics and detect emerging variants relevant to new RSV vaccines entering the market^[15-18].

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