The spatial-temporal dynamics of respiratory syncytial virus infections across the east–west coasts of Australia during 2016–17

Mark Robertson, 1, 4 John-Sebastian Eden, 2, 3, 4, 6 Avram Levy, 4, 5 Ian Carter, 1 Rachel L. Tulloch, 2, 3 Elena J. Cutmore, 2, 3 Bethany A. Horsburgh, 2, 3 Chisha T. Sikazwe, 4, 5 Dominic E. Dwyer, 1, 3 David W. Smith, 4, 5 and Jen Kok 1, 2

1 NSW Health Pathology-Institute for Clinical Pathology and Medical Research, NSW Health Pathology, Redbank Road, Westmead Hospital, Westmead, NSW 2145, Australia, 2 Centre for Virus Research, Westmead Institute for Medical Research, 176 Hawkesbury Road, Westmead, NSW 2145, Australia, 3 Marie Bashir Institute for Infectious Diseases and Biosecurity, Sydney Medical School, The University of Sydney, Sydney, NSW 2006, Australia, 4 Department of Microbiology, PathWest Laboratory Medicine WA, Hospital Ave, Nedlands, WA 6009, Australia, 5 School of Biological Sciences, The University of Western Australia, Crawley, WA 6009, Australia and 6 School of Medicine, The University of Western Australia, Crawley, WA 6009, Australia

Abstract

Respiratory syncytial virus (RSV) is an important human respiratory pathogen. In temperate regions, a distinct seasonality is observed, where peaks of infections typically occur in early winter, often preceding the annual influenza season. Infections are associated with high rates of morbidity and mortality and in some populations exceed that of influenza. Two subtypes, RSV-A and RSV-B, have been described, and molecular epidemiological studies have shown that both viruses mostly co-circulate. This trend also appears to be the case for Australia; however, previous genomic studies have been limited to cases from one Eastern state—New South Wales. As such, the broader spatial patterns and viral traffic networks across the continent are not known. Here, we conducted a whole-genome study of RSV comparing strains across eastern and Western Australia during the period January 2016 to June 2017. In total, 96 new RSV genomes were sequenced, compiled with previously generated data, and examined using a phylodynamic approach. This analysis revealed that both RSV-A and RSV-B strains were circulating, and each subtype was dominated by a single genotype, RSV-A ON1-like and RSV-B BA10-like viruses. Some geographical clustering was evident in strains from both states with multiple distinct sub-lineages observed and relatively low mixing across jurisdictions, suggesting that endemic transmission was likely seeded from imported, unsampled locations. Overall, the RSV phylogenies reflected a complex pattern of interactions across multiple epidemiological scales from fluid virus traffic across global and regional networks to fine-scale local transmission events.

Key words: respiratory syncytial virus; molecular epidemiology; Australia; whole-genome sequencing; phylogenetics

1. Introduction

Respiratory syncytial virus (RSV) is a major cause of acute respiratory tract infections in patients of all ages, producing significant morbidity and mortality (Shi et al., 2017). The greatest burden of disease is in children under 1 year old, where it is the most common cause of acute respiratory tract infection and in this age group is second only to malaria as a cause of death globally (Hall et al., 2009; Griffiths, Drews, and Marchant 2017). Importantly, RSV infection in young children may also lead to long-term sequelae such as asthma, chronic bronchitis, and obstructive pulmonary disease (Beigelman and Bacharier 2016; Griffiths, Drews, and Marchant 2017). Other populations particularly impacted by RSV include those over 65 years and immunosuppressed patients, such as solid organ and bone marrow transplant recipients (Beigelman and Bacharier 2016; Griffiths, Drews, and Marchant 2017).

Despite being discovered in the 1950s, the burden of RSV disease has only recently been appreciated, which is in part due to more reliable methods of diagnosis and detection. The laboratory diagnosis of RSV was initially reliant on viral isolation and the visualisation of characteristic syncytial cytopathic effects, for which its name is derived (Henrickson and Hall 2007). These techniques were slow and required technical expertise. The most commonly used modalities now include rapid antigen (such as lateral flow immunochromatography and fluorescent immunoassays) and nucleic acid amplification tests (NATs). Molecular assays, including both commercial and in-house NATs, offer increased sensitivity and specificity, as well as the ability to be multiplexed to detect other respiratory pathogens such as influenza, parainfluenza, rhinovirus, and human metapneumovirus (Mahony et al., 2007). Rapid test assays can also offer the additional benefit of early diagnosis, which allows for appropriate infection control.
interventions, rationalisation of unnecessary antibiotic therapy, and shorter hospitalisation periods (Henrickson and Hall 2007). More generally, improved diagnostics and reporting have begun to shed light on the true incidence, seasonal patterns, and peaks of activity of RSV. In temperate regions such as the southern major metropolitan areas of Australia, the seasonal peak typically occurs in the late autumn to early winter period (late-March to mid-August) in the months leading into the influenza season (Henrickson and Hall 2007; Di Giallonardo et al., 2018; Yeoh et al., 2020).

RSV can be divided into two antigenically and genetically distinct subtypes, RSV-A and RSV-B. These may be further divided into genetic groups based on the viral glycoprotein (G gene), termed genotypes, with at least 11 and 23 for RSV-A and RSV-B, respectively. However, recent work has proposed shifting to a genotype classification based on whole-genome sequencing (WGS) in order to increase phylogenetic resolution (Ramaekers et al., 2020). Serological studies have shown that the majority of people are infected by age 2, and whilst primary infections are important yet controversial topic. Studies have made associations of disease severity associated with RSV infection remains an et al., 2020.

2.1 Sample collection and processing

RSV can be co-circulating during a season and often at similar levels (Vandini, Biagi, and Lanari 2019); however, these are complicated by both host (Tal et al., 2004) and viral factors (DevVincenzo, El Saleebey, and Bush 2005), as well as their interactions. Molecular epidemiological studies have shown that both RSV-A and RSV-B co-circulate during a season and often at similar levels (Otiene et al., 2017; 2018; Pangesti et al., 2018; Park et al., 2017; Cattoir et al., 2019). Furthermore, for each of these subtypes, a single genotype will tend to predominate, such as with the recent RSV-A ON1-like and RSV-B BA10-like viruses (Eshaghi et al., 2012; Pretorius, Van Niekerk, and Tempia 2013; Di Giallonardo et al., 2018). Our understanding of the basic molecular epidemiology of RSV has been strengthened by WGS, which, similar to other pathogens, is becoming increasingly common in its application in infectious disease surveillance (Dapat et al., 2010; Agoti et al., 2014; Di Giallonardo et al., 2018). The added resolution from WGS has been particularly useful for elucidating transmission networks at both local (Agoti et al., 2015a) and epidemiological scales (Di Giallonardo et al., 2018), as well as for identifying and classifying the introduction and spread of new genotypes (Agoti et al., 2014; Ramaekers et al., 2020).

We recently performed the first genome-scale study of RSV molecular epidemiology in Australia (Di Giallonardo et al., 2018), demonstrating a wide diversity of co-circulating RSV lineages, with limited evidence of strong age and geographical clustering. However, this study was limited to cases obtained from eastern Australia in New South Wales (NSW) through the Western Sydney Local Health District. Here, we expand on these initial investigations to perform a transcontinental study of RSV genomic epidemiology in Australia. We compare RSV strains obtained from Western Australia (WA) to those from NSW on the east coast over an equivalent time period to examine the phylogenetic distribution of strains and to describe viral traffic between these regions.

2.2 Whole-genome sequencing

A previously published approach was used to amplify both RSV-A and RSV-B genomes (Di Giallonardo et al., 2018). In short, RT-PCR was used to amplify four overlapping amplicons (each ~4 kb) that together span the RSV genome. The size and yield of each RT-PCR was determined by agarose gel electrophoresis, and the four targets pooled equally. The pooled RSV amplicons were then purified with Agencourt AMPure XP beads (Beckman Coulter, USA) and quantified using the Quant-IT PicoGreen dsDNA Assay (Invitrogen, USA). The purified DNA was then diluted to 0.25 ng/µl and prepared for sequencing with the Nextera XT DNA library prep kit (Illumina, USA). Libraries were sequenced on an Illumina MiSeq using a 300 cycle v2 kit (150-nt paired-end reads). Raw paired sequence reads were trimmed using Trim Galore (https://github.com/FelixKrueger/TrimGalore; last accessed 11 April 2021) and then de novo assembled using Trinity (Grabherr et al., 2011). RSV contigs were identified by a local Blastn (Altschul et al., 1990) using a database of RSV reference genomes from NCBI RefSeq. The trimmed reads were remapped to draft genome contigs using BBMap (https://sourceforge.net/projects/bbmap/; last accessed 11 April 2021) to check the assembly, and the final majority consensus was extracted for each sample.

2.3 Phylogenetic analysis

The aim of this study was to compare the spatial and temporal dynamics of RSV infection in Australia. To do this, we used a phylogenetic approach to compare the distribution of strains circulating in two major, geographically distinct regions—NSW and WA—representing eastern Australia and WA, respectively, during the period from January 2016 to June 2017. All WA data, as well as NSW data from the year 2017, were generated within this study. To ensure even sampling across sites, these data were combined with NSW data from 2016 that was obtained from a previous study (Di Giallonardo et al., 2018). To provide additional context, RSV genome data was also sourced from NCBI GenBank where location (country) and collection date (year) was known. The Australian and global RSV genomes were first aligned with MAFFT (Katoh and Standley 2013), using the FFT-NS-i algorithm followed by manual inspection of gapped regions particularly in the G gene and non-coding regions. The alignments were then trimmed to include only the coding regions and screened for potential recombinants using RDP4 (Martin et al., 2015) with default parameters that were then removed before analysis. The RSV-A alignment included 1,190 sequences with a length of 15,747 nt, whilst the RSV-B alignment included 1,121 sequences with a length of 15,646 nt. To increase sampling resolution, we also generated comparable data sets
using the G gene region only that also included available partial-
genome sequences from NCBI GenBank that covered the G gene
region by at least 300 bp. The final G gene alignments contained
6,603 and 4,300 sequences for RSV-A and RSV-B, respectively,
and were both trimmed to the G gene coding region (approximately 960
bp long). The best-fit DNA substitution model for these data was
determined with jModelTest (Darriba et al., 2012). Maximum like-
lihood (ML) trees were then estimated for both RSV-A and RSV-B
alignments using RAxML (Stamatakis 2014) employing the best-
fit model, which in both cases was found to be the General-Time
Reversible model with a gamma distribution of rates (GTR + G).
Support for individual nodes was determined by 1,000 bootstrap
replications.

2.4 Phylogeographic clustering
From our overall ML trees, it was found that all sequences from
2016 and 2017 were either RSV-A ON1-like or RSV-B BA10-like
viruses; therefore, we limited further analyses to these specific
clusters. To evaluate the geographic structure in the RSV-A and
RSV-B trees, we used the Bayesian Tip-association Significance
(BaTS) program (Parker, Rambaut, and Pybus 2008), with 1,000
replicates to analyse the genome data sets. Using BaTS, we deter-
mined the parsimony score (PS), association index (AI), and max-
imum clade size statistics for the location associated with each
sequence, specifically focusing on the comparison between east-
ern Australia (NSW) and WA. To account for other jurisdictions (countries) present
in the data, such sequences were assigned to their continent of
sampling. This analysis required a posterior distribution of trees,
which were obtained using the Bayesian Markov Chain Monte
Carlo method implemented in BEAST v1.10.4 (Drummond et al.,
2012). Here, we used the best-fit DNA model (GTR + G) with a strict
clock and a constant population size, as shown to be appropriate
previously (Di Giallonardo et al., 2018). All analyses were run for
50 million steps, with sampling every 500 steps with 10–20 per cent
burn-in. To ensure convergence, three independent runs were
conducted and merged to obtain the final set of trees used in the
clustering analysis. The maximum clade credibility tree from the
Bayesian analyses using BEAST was generated with heights scaled
to mean values.

2.5 Local transmission events
In order to identify potential local transmission events (for
instance, an outbreak at an institution), we examined both ML and
time-scaled Bayesian trees using the genome data sets, for mono-
phyletic groups containing near-identical sequences (~99.9 per
cent nucleotide identity, less than 10 base pairs different across
the genome) sampled up to 2 weeks apart. Patient demograph-
ics and sampling location were then mapped to determine traits
associated with these fine-scale phylogenetic groups. The G gene
ML trees were then used to validate clustering and to consider
additional sources (sampling locations) for local RSV cases.

2.6 Data availability
All sequences generated in this study have been submitted
to NCBI GenBank (MW160744-MW160839). Furthermore, data
and material relevant to this study are available from https://
github.com/jsede/RSV_NSW_WA (last accessed 17 April 2021).

3. Results and discussion
3.1 Whole-genome sequencing of RSV strains
from NSW and WA in 2016 and 2017
In order to compare the spatial distribution of RSV strains across
eastern Australia (NSW) and WA Australia during the period from
January 2016 to June 2017, we performed WGS on stored samples
collected from community and hospitalised persons presenting
with an influenza-like illness. The demographic details of the
cohort have been summarised in Table 1. For the NSW cases,
nom specific sample selection criteria were used except for sample
availability from an archived collection of RSV-positive residual
diagnostic specimens. Most samples from NSW were obtained
from children ≤5 years of age (51.6 per cent, n = 65/126), with a
further 20.6 per cent of samples collected from patients ≥65 years
(n = 26/126). In contrast, samples from WA were selected to repre-
sent all age groups. Despite this, similar to NSW, most available
samples were collected from the young (62.1 per cent, n = 64/103
from patients ≤5 years of age) and the elderly (17.5 per cent,

Table 1. Demographic details of NSW and WA RSV cohorts.

| Age (years) | NSW | WA |
|------------|-----|----|
| ≤5         | 65 (51.6) | 64 (62.1) |
| 5–65       | 35 (27.8) | 21 (20.4) |
| ≥65        | 26 (20.6) | 18 (17.5) |
| Total      | 126 (100%) | 103 (100%) |

| Sex | NSW | WA |
|-----|-----|----|
| Male | 66 (52.4) | 48 (46.6) |
| Female | 60 (47.6) | 55 (53.4) |
| Total | 126 (100) | 103 (100) |

![Figure 1. WGS of RSV in WA and NSW between January 2016 and June 2017 by month. The y-axis represents counts of genomes sequenced. The x-axis represents months when the samples were collected, with year shown underneath. RSV-A and RSV-B subtypes have been coloured blue and red respectively, as per the key provided. In total, over the period of investigation, 189 RSV genomes were compared, including 63 from WA and 126 from NSW. WA samples were selected pre-sequencing to cover both subtypes during seasonal and inter-seasonal periods and therefore do not necessarily reflect the subtype or temporal distribution of RSV in WA. The asterisk indicates that of the 126 NSW genomes, 93 are from patients ≤5 years of age, and the remaining 33 are from ≥65 years of age.](https://github.com/jsede/RSV_NSW_WA)
n = 18/103 from patients ≥65 years), reflecting the distribution of infection at both extremes of age. The distribution between sexes was approximately equal for both study sites (Table 1).

Virus genomes were successfully extracted and sequenced from 63 of the 103 respiratory samples collected in WA during 2016 and 2017, and 32 of the 45 NSW cases collected in 2017 (total new genomes n = 96, where one case from NSW was an RSV-A/B mixed infection). These data were combined with existing genomes from NSW during 2016 (n = 93) generated previously (Di Giallonardo et al., 2018), bringing the total number of Australian genomes for analysis across both states in 2016 and 2017 in the current study to 189. The breakdown between sampling location and RSV subtypes determined by WGS shows that both RSV-A and RSV-B subtypes were present in both NSW and WA populations (Fig. 1). In NSW, there was a higher proportion of RSV-B strains (63 per cent, n = 80/126), which was consistent across the entire study period. Furthermore, in NSW, the peak in RSV activity typically occurs in May–June each year (NSW Health influenza surveillance data); however, most of our genome sequences in 2016 were from isolates in August–October that year, that is, they were from the later part of the RSV season. In WA, there was even representation of RSV-A and RSV-B in the WGS data across 2016, with an increase in the relative proportion of RSV-A during 2017. Whilst our sampling for WA was intentionally even with regard to time, subtype, age, and setting, the predominance of RSV-A during 2017 was observed by the initial diagnostic testing where the assay used resolves RSV subtypes. Specifically, with this lab testing data, a transition from RSV-B to RSV-A was observed between the 2016 and 2017 seasons in WA (data not shown). These results are consistent with other molecular epidemiological studies globally that show both RSV-A and RSV-B subtypes co-circulate with shifting predominance across seasons (Di Giallonardo et al., 2018; Luo et al., 2020; Razanajatovo Rahombananahary et al., 2020; Yun, Choi, and Lee 2020).
3.2 Phylodynamics of RSV infections in NSW and WA in a global context

The WGS data from NSW and WA were aligned and compared to global genome references to provide genetic context for local strains. Phylogenetic analysis using a ML approach was then employed to examine the diversity of RSV-A and RSV-B strains (Figs 2 and 3). This analysis revealed that for each subtype, regardless of location, a single genotype was predominant. For RSV-A, recent viruses from both NSW and WA were derived from the ON1 lineage (Eshaghi et al., 2012), which has been the predominant RSV-A genotype globally since it first emerged in 2011 (Eshaghi et al., 2012; Agoti et al., 2014; Tabatabai et al., 2014; Di Giallonardo et al., 2018) (Fig. 2). Similarly, the RSV-B phylogeny showed that circulating viruses were mostly of the BA10 lineage (Fig. 3). From the limited available sequence data on GenBank, it appears that the BA10-like viruses are circulating globally; however, there are few published molecular epidemiology studies to support the suggestion they are predominant. It is clear, however, that in Australia, in both NSW and WA that this has been the major RSV-B genotype since at least 2014 (Di Giallonardo et al., 2018).

Across both RSV-A and RSV-B phylogenies, there does not appear to be strict spatial clustering when comparing viruses from NSW and WA (Figs 2 and 3), that is, the viruses from WA and NSW do not form monophyletic groups. Such monophyletic clustering would not be expected based on what is known for other respiratory pathogens—such as influenza—which are characterised by high levels of gene flow and viral traffic at global scales (Rambaut et al., 2008; Vijaykrishna et al., 2015). As such, we observed multiple co-circulating sub-lineages distributed across the entire diversity of RSV-A ON1-like and RSV-B BA10-like viruses (Figs 2 and 3). However, within these sub-lineages, the viruses often formed clusters based on sampling location; therefore, local spatial structure was apparent in our analysis. To explore this formally, we conducted a clustering analysis with BaTS and a posterior set of trees estimated using a time-scaled phylogenetic analysis in BEAST. We limited the analysis to the RSV-A ON1-like
and RSV-B BA10-like viruses, and the maximum clade credibility tree for each RSV subtype was found to be congruent with the ML trees (Figs 2 and 3). Using the posterior set of trees, we then measured the degree of clustering based on sampling location (Table 2).

When sequences were grouped by broad geographical regions, specifically, continents and the two Australian states NSW and WA (and VIC for RSV-A data), phylogeny-trait association tests indicated a significant pattern of overall geographical clustering (P-values <0.001) as measured by AI and PS (Table 2). For individual locations, most were found to cluster with significant scores. This includes Asia, the Americas, the Middle East, Africa, Oceania, NSW, WA, and VIC for RSV-A viruses (all P-values <0.008) and then similarly all for RSV-B where the sampling was sufficient (n >4 sequences). This analysis demonstrates that, at larger epidemiological scales, viral lineages are often imported and established locally. We note, however, that this analysis is biased for regions that have been highly sampled, as highlighted by the American and Kenyan viruses, which are mostly from one specific city and/or hospital (Supplementary Figs S1 and S2) (Otieno et al., 2018). Our sampling of NSW and WA strains is similarly constrained, except that our sampling sites are both major population centres on the east coast such as Sydney and Melbourne. Our sampling of NSW and WA strains is similarly constrained, except that our sampling sites are both major population centres on the east coast such as Sydney and Melbourne. Our sampling of NSW and WA strains is similarly constrained, except that our sampling sites are both major population centres on the east coast such as Sydney and Melbourne.

Table 2. Phylogeny-trait association test for RSV in Australia and other regions globally.

| Location        | RSV-A | RSV-B |
|-----------------|-------|-------|
| Overall clustering | 499   | 470   |
| Asia            | 24    | 26    |
| South America   | 16    | 42    |
| North America   | 185   | 17    |
| Middle East     | 5     | 0     |
| Oceania         | 28    | 2     |
| Africa (NSW)    | 147   | 263   |
| Australia (VIC) | 2     | 0     |
| Australia (WA)  | 31    | 32    |
| Europe          | 1     | 4     |

P-values <0.001 as measured by AI and PS.

| Location        | RSV-A | RSV-B |
|-----------------|-------|-------|
| Overall clustering | 6     | 4     |
| Asia            | 28    | 2     |
| South America   | 147   | 263   |
| North America   | 31    | 32    |
| Middle East     | 1     | 4     |

P-values <0.001 as measured by AI and PS.

Next, we examined genetic clusters defined by high genetic identity (<10-bp difference across the genome) and similar sampling periods (collection dates within 2 weeks) to consider what features such as patient age and sampling location define them. For RSV-A, we identified 8 clusters (Supplementary Fig. S1 and Table S1), and for RSV-B, we identified 10 clusters (Supplementary Fig. S2 and Table S2). In this study, 75 per cent (n = 6/8) and 80 per cent (n = 8/10) of RSV-A and RSV-B clusters, respectively, could be linked to common localities and/or institutions, including individual hospital wards or emergency departments. While two of the RSV-B clusters were found to be repeat samples of the same patient (Supplementary Table S2), overwhelmingly, these clusters of genetically and temporally related sequences most likely represent fine-scale transmission events.

4. Conclusions

In summary, our analysis has identified a number of features of RSV epidemiology and patterns of spread, including (1) the co-circulation of both major subtypes—RSV-A and RSV-B, (2) a single genotype for each subtype predominates each season, (3) multiple distinct sub-lineages of each genotype will co-circulate and which are associated with regional and local clustering/outbreaks,
(4) little viral mixing across the east–west coasts of Australia despite apparent overall geographic clustering, (5) that genetically and temporally-related sequences most likely represent fine-scale transmission events such as institutional outbreaks, and (6) that whole-genome sequence is required and encouraged over partial G gene sequencing for elucidating clusters and transmission pathways. Taken together, this presents a complex phylogeographic pattern with globally circulating diversity with viral mixing across different regions, yet, finer-scale patterns revealing multiple endemic sub-lineages and clusters consistent with local transmission events and outbreaks. This highlights the connections of genomic data across multiple epidemiological scales and further strengthens the need for a much greater sampling of RSV, not just here in Australia, but globally.

**Supplementary data**  
Supplementary data is available at *Virus Evolution* online.

**Acknowledgements**  
We thank all the members of the Virology and Microbiology teams at NSW Health Pathology—ICPMR in Westmead, NSW, and the PathWest QE2 Medical Centre laboratories in Perth, WA, for all their contributions towards processing the diagnostics specimens used in this study.

**Funding**  
Funding was provided through the Institute for Clinical Pathology and Medical Research (ICPMR) Private Practice Trust fund, the National Health and Medical Research Council Centre of Research Excellence in Emerging Infectious Diseases (1102962) and the Marie Bashir Institute for Infectious Diseases and Biosecurity at the University of Sydney.

**Conflict of interest:** None declared.

**References**  
Agot, C. N. et al. (2014) ‘Rapid Spread and Diversification of Respiratory Syncytial Virus Genotype ON1, Kenya’, *Emerging Infectious Diseases*, 20: 950–9.

——— et al. (2015a) ‘Local Evolutionary Patterns of Human Respiratory Syncytial Virus Derived from Whole-Genome Sequencing’, *Journal of Virology*, 89: 3444–54.

——— et al. (2015b) ‘Successive Respiratory Syncytial Virus Epidemics in Local Populations Arise from Multiple Variant Introductions, Providing Insights into Virus Persistence’, *Journal of Virology*, 89: 11630–42.

Altschul, S. F. et al. (1990) ‘Basic Local Alignment Search Tool’, *Journal of Molecular Biology*, 215: 403–10.

Beigelman, A., and Bacharier, L. B. (2016) ‘Early-Life Respiratory Infections and Asthma Development: Role in Disease Pathogenesis and Potential Targets for Disease Prevention’, *Current Opinion in Allergy and Clinical Immunology*, 16: 172–8.

Cattoir, L. et al. (2019) ‘Epidemiology of RSV and hMPV in Belgium: A 10-Year Follow-up’, *Acta Clinica Belgica*, 74: 229–35.

Dapat, I. C. et al. (2010) ‘New Genotypes within Respiratory Syncytial Virus Group B Genotype BA in Niigata, Japan’, *Journal of Clinical Microbiology*, 48: 3423–7.

Darriba, D. et al. (2012) ‘jModelTest 2: More Models, New Heuristics and Parallel Computing’, *Nature Methods*, 9: 772.

DeVincenzo, J. P., El Saleeby, C. M., and Bush, A. J. (2005) ‘Respiratory Syncytial Virus Load Predicts Disease Severity in Previously Healthy Infants’, *The Journal of Infectious Diseases*, 191: 1861–8.

Di Giannolando, F. et al. (2018) ‘Evolution of Human Respiratory Syncytial Virus (RSV) over Multiple Seasons in New South Wales, Australia’, *Viruses*, 10: 476.

Drummond, A. J. et al. (2012) ‘Bayesian Phylogenetics with BEAUti and the BEAST 1.7’, *Molecular Biology and Evolution*, 29: 1969–73.

Eshaghi, A. et al. (2012) ‘Genetic Variability of Human Respiratory Syncytial Virus A Strains Cirulating in Ontario: A Novel Genotype with A 72 Nucleotide G Gene Duplication’, *PLoS One*, 7: e32807.

Geoghegan, J. L. et al. (2018) ‘Continental Synchronicity of Human Influenza Virus Epidemics despite Climatic Variation’, *PloS Pathogens*, 14: e1006780.

Grabherr, M. G. et al. (2011) ‘Full-length Transcriptome Assembly from RNA-Seq Data without a Reference Genome’, *Nature Biotechnology*, 29: 644–52.

Griffiths, C., Drews, S. J., and Marchant, D. J. (2017) ‘Respiratory Syncytial Virus: Infection, Detection, and New Options for Prevention and Treatment’, *Clinical Microbiology Reviews*, 30: 277–319.

Hall, C. B. et al. (2009) ‘The Burden of Respiratory Syncytial Virus Infection in Young Children’, *The New England Journal of Medicine*, 360: 588–98.

Henrickson, K. J., and Hall, C. B. (2007) ‘Diagnostic Assays for Respiratory Syncytial Virus Disease’, *The Pediatric Infectious Disease Journal*, 26: S36–40.

Katoh, K., and Standley, D. M. (2013) ‘MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability’, *Molecular Biology and Evolution*, 30: 772–80.

Luo, H.-J. et al. (2020) ‘Epidemiological Characteristics and Phylogenetic Analysis of Human Respiratory Syncytial Virus in Patients with Respiratory Infections during 2011–2016 in Southern China’, *International Journal of Infectious Diseases: IJID: Official Publication of the International Society for Infectious Diseases*, 90: 5–17.

Mahony, J. et al. (2007) ‘Development of a Respiratory Virus Panel Test for Detection of Twenty Human Respiratory Viruses by Use of Multiplex PCR and a Fluid Microbead-based Assay’, *Journal of Clinical Microbiology*, 45: 2965–70.

Martin, D. P. et al. (2015) ‘RDP4: Detection and Analysis of Recombination Patterns in Virus Genomes’, *Virus Evolution*, 1: vey003.

Otierno, J. R. et al. (2017) ‘Spread and Evolution of Respiratory Syncytial Virus A Genotype ON1, Coastal Kenya, 2010–2015’, *Emerging Infectious Diseases*, 23: 264–71.

——— et al. (2018) ‘Whole Genome Analysis of Local Kenyan and Global Sequences Unravels the Epidemiological and Molecular Evolutionary Dynamics of RSV Genotype ON1 Strains’, *Virus Evolution*, 4: vey027.

Pangesti, K. N. A. et al. (2018) ‘Molecular Epidemiology of Respiratory Syncytial Virus’, *Reviews in Medical Virology*, 28: e1968.

Park, E. et al. (2017) ‘Molecular and Clinical Characterization of Human Respiratory Syncytial Virus in South Korea between 2009 and 2014’, *Epidemiology and Infection*, 145: 3226–42.

Parker, J., Rambaut, A., and Pybus, O. G. (2008) ‘Correlating Viral Phenotypes with Phylogeny: Accounting for Phylogenetic Uncertainty’, *Infection, Genetics and Evolution: Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases*, 8: 239–46.

Pretorius, M. A., van Niekerk, S., and Tempia, S. (2013) ‘Replacement and Positive Evolution of Subtype A and B Respiratory Syncytial Virus G-protein Genotypes from 1997–2012 in South Africa. The Journal of Infectious Diseases’. <https://academic.oup.com/jid/article-abstract/208/suppl_3/S227/789685> Accessed 21 Jul 2021.
Ramaekers, K. et al. (2020) ‘Towards a Unified Classification for Human Respiratory Syncytial Virus Genotypes’, Virus Evolution, 6: veaa052.

Rambaut, A. et al. (2008) ‘The Genomic and Epidemiological Dynamics of Human Influenza A Virus’, Nature, 453: 615–9.

Razanajatovo Rahombanjanahary, N. H. et al. (2020) ‘Genetic Diversity and Molecular Epidemiology of Respiratory Syncytial Virus Circulated in Antananarivo, Madagascar, from 2011 to 2017: Predominance of ON1 and BA9 Genotypes’, Journal of Clinical Virology: The Official Publication of the Pan American Society for Clinical Virology, 129: 104506.

Shi, T. et al. (2017) ‘Global, Regional, and National Disease Burden Estimates of Acute Lower Respiratory Infections Due to Respiratory Syncytial Virus in Young Children in 2015: A Systematic Review and Modelling Study’, The Lancet, 390: 946–58.

Stamatakis, A. (2014) ‘RAxML Version 8: A Tool for Phylogenetic Analysis and Post-analysis of Large Phylogenies’, Bioinformatics, 30: 1312–3.

Tabatabai, J. et al. (2014) ‘Novel Respiratory Syncytial Virus (RSV) Genotype ON1 Predominates in Germany during Winter Season 2012–13’, PLoS One, 9: e109191.

Tal, G. et al. (2004) ‘Association between Common Toll-like Receptor 4 Mutations and Severe Respiratory Syncytial Virus Disease’, The Journal of Infectious Diseases, 189: 2057–63.

Vandini, S., Biagi, C., and Lanari, M. (2017) ‘Respiratory Syncytial Virus: The Influence of Serotype and Genotype Variability on Clinical Course of Infection’, International Journal of Molecular Sciences, 18: 1717.

Vijaykrishna, D. et al. (2015) ‘The Contrasting Phylodynamics of Human Influenza B Viruses’, eLife, 4: e05055.

Yeoh, D. K. et al. (2020) ‘The Impact of COVID-19 Public Health Measures on Detections of Influenza and Respiratory Syncytial Virus in Children during the 2020 Australian Winter’, Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America, 72: 2199–202.

Yun, K. W., Choi, E. H., and Lee, H. J. (2020) ‘Molecular Epidemiology of Respiratory Syncytial Virus for 28 Consecutive Seasons (1990–2018) and Genetic Variability of the Duplication Region in the G Gene of Genotypes ON1 and BA in South Korea’, Archives of Virology, 165: 1069–77.

Zou, L. et al. (2016) ‘Evolution and Transmission of Respiratory Syncytial Group A (RSV-A) Viruses in Guangdong, China 2008–2015’, Frontiers in Microbiology, 7: 1263.