


Original Article

Travel-associated SARS-CoV-2 transmission documented with whole genome sequencing following a long-haul international flight

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Abstract

Background: Multiple instances of flight-associated severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) transmission during long-haul flights have been reported during the COVID-19 pandemic. However, comprehensive investigations of passenger risk behaviours, before, during and after the flight, are scarce.

Methods: To investigate suspected SARS-CoV-2 transmission during a flight from United Arab Emirates to Australia in July 2020, systematic, repeated polymerase chain reaction (PCR) testing of passengers in hotel quarantine was linked to whole genome sequencing. Epidemiological analyses of in-depth interviews covering behaviours during the flight and activities pre- and post-boarding were used to identify risk factors for infection.

Results: Seventeen of the 95 passengers from four different travel origins had PCR-confirmed infection yielding indistinguishable genomic sequences. Two of the 17 passengers were symptomatic within 2 days of the flight, and classified as co-primary cases. Seven secondary cases were seated within two rows of the co-primary cases, but five economy passengers seated further away and three business class passengers were also infected (attack rate = 16% [15/93]). In multivariable analysis, being seated within two rows of a primary case [odds ratio (OR) 7.16; 95% confidence interval (CI) 1.66–30.85] and spending more than an hour in the arrival airport (OR 4.96; 95% CI 1.04–23.60) were independent predictors of secondary infection, suggesting travel-associated SARS-CoV-2 transmission likely occurred both during and after the flight. Self-reported increased hand hygiene, frequent aisle walking and using the bathroom on the plane did not independently affect the risk of SARS-CoV-2 acquisition.

Conclusions: This investigation identified substantial in-flight transmission among passengers seated both within and beyond two rows of the primary cases. Infection of passengers in separate cabin classes also suggests transmission occurred outside the cabin environment, likely at the arrival airport. Recognizing that transmission may occur pre- and post-boarding may inform contact tracing advice and improve efforts to prevent future travel-associated outbreaks.

Key words: COVID-19, SARS-CoV-2, disease outbreaks, air travel, pandemics

Introduction

As COVID-19 vaccination programs are implemented around the world, countries have reopened their borders to international air travel. To mitigate the further spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) on flights, risk factors and potential transmission routes for infection associated with air travel need to be identified and managed. There are multiple points during international air travel where SARS-CoV-2 transmission could occur including during check-in and boarding at the departure airport; while in-flight; at disembarkation; during immigration processing and baggage collection at arrival airports and during transport to accommodation. Although an increasing number of in-cabin SARS-CoV-2 transmission events during long-haul flights have been reported,¹⁻⁴ and the potential role of contact outside the cabin environment has been acknowledged,^{3,5-7} comprehensive investigations of potential SARS-CoV-2 exposures occurring pre- and post-flight are limited.

On 1 July 2020, a 354 seat Boeing 777 landed at Perth, Australia, after departing Dubai, United Arab Emirates (UAE) 10 hours earlier with 95 passengers on-board. Ninety of the passengers were placed into 14 days of mandatory quarantine and underwent periodic testing for SARS-CoV-2. Twenty passengers developed laboratory-confirmed SARS-CoV-2 infection while in quarantine, prompting an investigation into potential flight-associated transmission using whole genome sequencing (WGS) and structured passenger interviews. We report on the outbreak investigation with the aim to identify behaviours and situations potentially associated with SARS-CoV-2 exposure before, during and after the flight, and to quantify the risks associated with air travel.

Methods

Quarantine requirements for international arrivals

From March 2020 until February 2022, all international passengers arriving into Western Australia (WA) were required to undergo a 14-day quarantine period in state-managed hotels, unless an exemption had been granted.⁸ International arrivals were transported from the airport to quarantine hotels by a government-contracted bus service. During the period of this investigation, all individuals in hotel quarantine were required to have a nasopharyngeal swab tested for SARS-CoV-2 on Day 2 and Day 12 after arrival. Quarantined travellers were also tested any time they reported symptoms compatible with COVID-19.

Data collection

Structured interviews with passengers were conducted between 6 August and 29 September 2020 to ascertain the country where travel originated, seat location, movement around the cabin, hand hygiene and mask use behaviours before, during and after the flight, and transit times (Appendix 1). At least four attempts were made to contact all passengers via telephone or email and 79% (75/95) were successfully interviewed. Of those not interviewed, 5 declined, 11 did not respond to attempts to contact them, 3 had incorrect contact details and 1 was a minor who

had not travelled with a parent/guardian. Responses were entered onto a REDCap database (2020, Vanderbilt University).

Polymerase chain reaction (PCR) testing, WGS and culture methods were performed as previously described.⁹ To characterize the genomics of this outbreak, WGS was attempted on all PCR-positive specimens. WA genome sequences of SARS-CoV-2 were assigned lineages using the Phylogenetic Assignment of Named Global Outbreak LINEages (PANGOLIN) tool. SARS-CoV-2 complete genomes with corresponding metadata were retrieved from the Global Initiative on Sharing All Influenza Data database on 13 December 2021. The final dataset contained 381 WGS that were aligned with the WA sequences generated in this study using the MAFFT v7.467 software. Phylogenetic trees were visualized using the software package Figtree v1.4.4. Laboratory scientists overseeing or performing the WGS were blinded to the epidemiological findings at the time of sequencing and data analysis. Culture was attempted on all samples.

The airline provided information regarding the plane's air filtration system; check-in, boarding and disembarkation procedures specific to the flight; required use of personal protective equipment by passengers and flight attendants; flight attendant lavatory use; and the passenger manifest and seating map.

Definitions

The likely source of infection for passengers positive for SARS-CoV-2 was determined using a combination of epidemiological and laboratory information including contact tracing interviews, passenger questionnaire responses, WGS information for individuals and in countries of travel origin, specimen cycle threshold (Ct) values and culture results.

Passengers were categorized into four groups for this analysis. Primary cases were defined as passengers who (i) reported symptom onset during or within 2 days of the flight, (ii) were PCR-positive for SARS-CoV-2 on Day 2, (iii) had a specimen sequence within the genomic cluster and (iv) travelled from a country where the genomic sequences associated with this cluster could have originated. Passengers were defined as secondary cases if they (i) were PCR-positive for SARS-CoV-2 during quarantine and (ii) had a specimen sequence within the genomic cluster, or a symptomatic individual with PCR-confirmed infection whose specimen did not yield sufficient sequence data but shared a hotel room with another secondary case with a WGS in the genomic cluster. Passengers were considered unlinked cases if they were PCR-positive for SARS-CoV-2 while in quarantine, but their genome sequence was not in the genomic cluster. Passengers who were PCR-negative for SARS-CoV-2 on two or more specimens collected during quarantine were considered non-cases.

Data analysis

The reported frequency of mask use, hand washing, hand sanitizer use, glove use, eating and drinking, time at Dubai airport, Perth airport and on the bus from Perth airport to hotel quarantine were examined for all passengers. Movement up and down the plane aisle, in-flight bathroom use and other measures taken to reduce exposure to SARS-CoV-2 on the plane were examined for economy class passengers only as this is where the primary

cases were seated. The response of wearing a mask ‘all of the time’ permitted exceptions for eating, drinking and passport control. Mask wearing ‘not all the time’ included responses of ‘most of the time’, ‘some of the time’ and ‘none of the time’. Responses were analysed using risk ratios and Fisher’s exact tests, as was infection risk by seating location. Exposures with $P \leq 0.05$ were considered significantly associated with the risk of being a secondary case and included in a multivariate logistic regression model using STATA v15 software.

Ethics considerations

Ethics approval was obtained from the Australian National University Human Ethics committee, protocol number 2021/391.

Results

Ninety-five passengers travelling from 11 different countries boarded the flight in Dubai; 3 were seated in first class, 15 in business and 77 in economy. Passengers reported mandatory mask wearing in the UAE, including at Dubai airport, which was described as uncharacteristically empty, with travellers socially distancing prior to the flight. Masks were also mandatory on the flight from Dubai to Perth, unless the passenger had a medical exemption or was a child under 6 years of age.¹⁰ Travel packs including masks, gloves, hand sanitizer and cleaning wipes were provided to each passenger by the airline. Flight-crew wore masks, eye goggles, gloves and a protective gown at all times (communication from airline, January 2021). The in-cabin air filtration system on this flight was reported to be functioning correctly (communication from airline, January 2021). Upon arrival in Perth, passengers progressed through immigration, baggage claim and customs, as well as a state-run illness checkpoint. Five passengers with exemptions self-quarantined at another location and routine testing was not conducted on these passengers. No other flights arrived at the Perth international airport on the same day.

Six passengers tested PCR-positive for SARS-CoV-2 on Day 2 after arrival, and a further 14 infections were identified later in the quarantine period (Figure 1). All cases were interviewed by contact tracers to investigate potential exposures and symptoms. None of the passengers with SARS-CoV-2 infection were hospitalized or died. Flight crew were not part of the public health investigation at that time, but subsequent communication with the airline revealed no infections among flight crew who travelled on the flight.

WGS was attempted on all 20 specimens; 8 yielded complete genomes and 10 partial genomes sufficient to be included in a phylogenetic analysis. Lineage assessment of these 18 SARS-CoV-2 genomes revealed the circulation of the B.1.480 ($n = 17$, 94%) lineage and a distinct B.1 lineage ($n = 1$, 6%). For the two remaining specimens, one yielded enough WGS information to be excluded from the B.1.480 cluster and the other sequence was insufficient for analysis. The B.1.480 lineage was detected in passengers with originating travel from the UK, Ethiopia, France and the UAE. At the time of the flight, the prevalence of B.1.480 lineage was extremely low (<0.01%) from these countries. As of 13 December 2021, this lineage represents <0.00015% of global SARS-CoV-2 sequences uploaded to GISAID. Thirty percent

(7/25) of all SARS-CoV-2 samples from Ethiopia sequenced have been assigned the B.1.480 lineage.

To determine relatedness and origins of the outbreak lineage, a maximum likelihood tree was generated from complete or near complete genomes from this investigation, together with all the available B.1.480 SARS-CoV-2 genomes uploaded to GISAID as of 13 December 2021 ($n = 381$). This analysis revealed that all the B.1.480 genomes from this study branched into a well-supported monophyletic cluster (B.1.480-EK) (Figure 2), but none of the available global sequences uploaded to GISAID nested within the B.1.480-EK cluster.

Two passengers (passenger 1 and passenger 3), who were related and over 60 years old, were identified as co-primary cases. Both had travelled together from Ethiopia to board the flight in Dubai; both had Day-2 specimens yielding the B.1.480 lineage which clustered with sequences obtained from other passengers. One of the primary cases (passenger 3) was symptomatic on the flight and the other developed symptoms within 2 days of arrival in Perth and therefore considered potentially infectious *en route* (Figure 1).

Fifteen passengers were identified as secondary cases yielding a secondary attack rate of 16% (15/93). The ages of the secondary cases ranged from 1 to 56 years (median 36 years), three of which were children <6 years of age and not subject to the mask mandate; eight (60%) were male. Fourteen of the secondary cases had SARS-CoV-2 infection during quarantine, each comprising genomes belonging to the B.1.480-EK cluster. The remaining secondary case was a child with PCR-confirmed infection for whom sequencing was unsuccessful but whose parents had genomic sequences in the cluster. Symptom onset among the eight secondary cases with illness ranged from 3 to 12 days following the flight and included cough, sore throat, fever, body ache, headache, chest tightness, shortness of breath and nasal congestion. Seven of the secondary cases were asymptomatic during quarantine but PCR-positive on Day-12 specimens (Figure 1).

Secondary cases were identified among seven distinct travelling parties (two individuals and five separate family groups), commencing their journeys from the UAE, UK and France. Single-nucleotide polymorphism (SNP) variation for passengers 5, 6 and 7 was supportive of familial transmission. Three other passengers were classified as unlinked cases. One of these individuals (passenger 2) had travelled from Ethiopia and whose specimen yielded a B.1.480 genomic sequence with unique SNP mutations not shared by any other sequenced specimens (Figure 2). This individual had a Day-2 PCR-positive specimen with a high Ct value (39.5) which was culture negative, and combined with their clinical history, was suggestive of prior infection in Ethiopia. The other two unlinked cases also had Ct values above 35 on Day 2; one was obtained from a passenger (passenger 4) who originally travelled from Afghanistan and was infected with a B.1 lineage virus that did not belong to the B.1.480-EK cluster (Figure 2). The other passenger commenced their journey in Romania and their specimen provided limited, but sufficient genomic data to be considered distinct from the B.1.480-EK cluster.

The seating location of passengers on the flight, by case classification, is shown in Figure 3. In univariate analyses, passengers seated within two rows of the primary cases were at 2.71 [95%

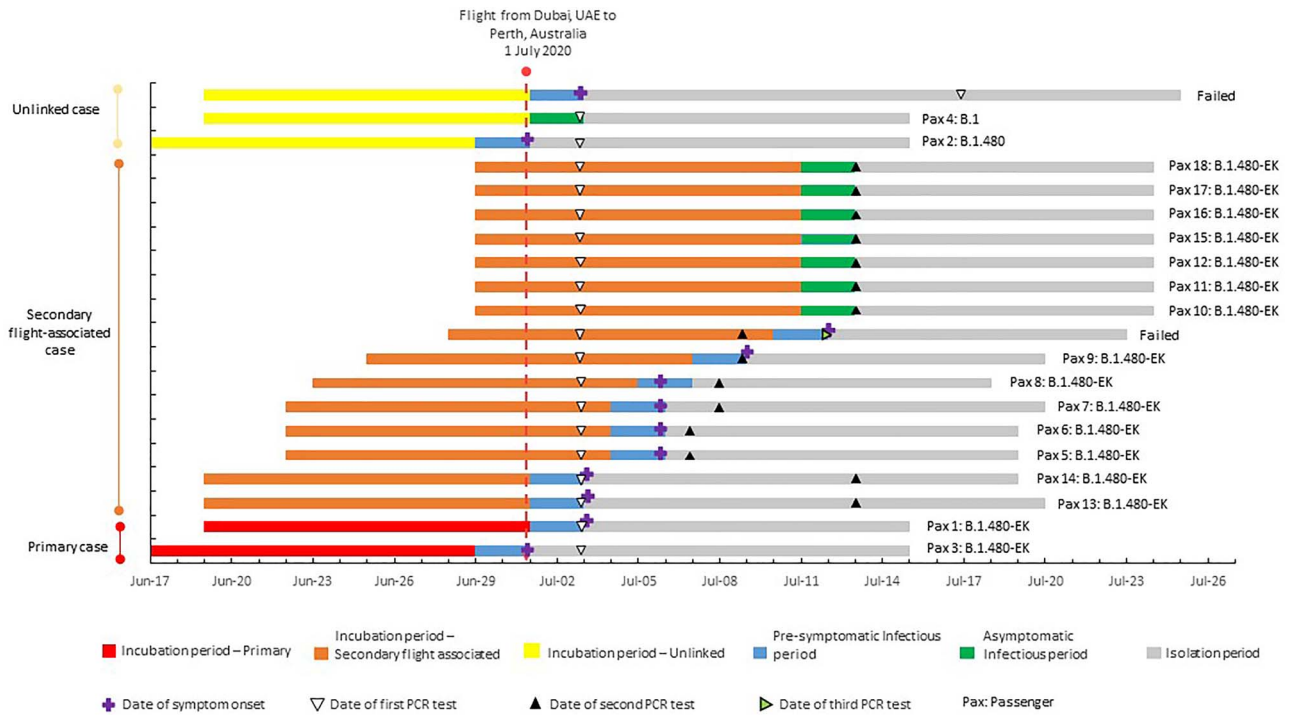


Figure 1. Epidemiologic and clinical timeline for passengers on flight from Dubai to Perth, 1 July 2020. SARS-CoV-2 lineage determined by WGS.

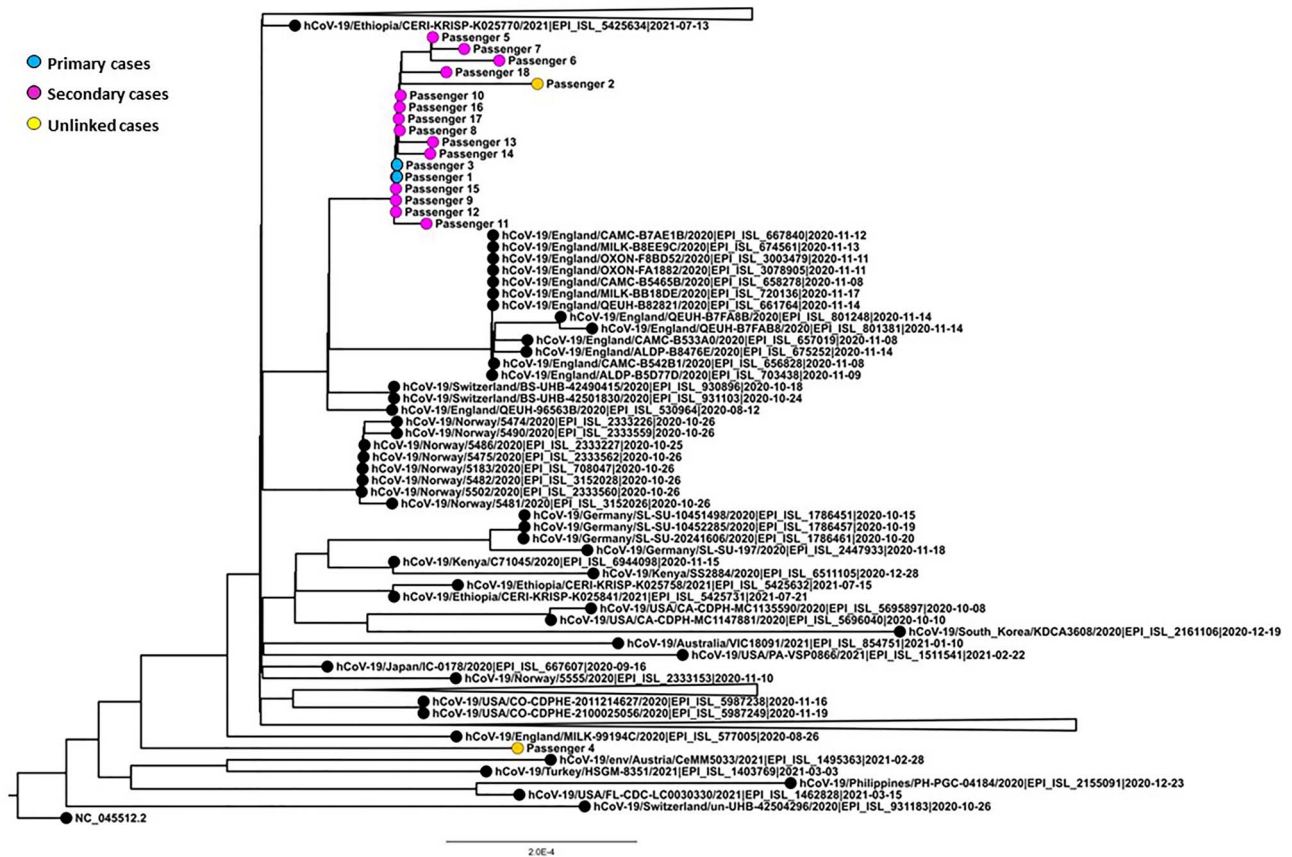


Figure 2. A global maximum likelihood phylogenetic tree* including the B.1.480 lineage from genomes submitted to GISAID as of 13 December 2021. *All branch lengths are drawn to a scale of nucleotide substitutions per site and the tree is rooted to the prototype strain of SARS-CoV-2 (NC_045512.2).

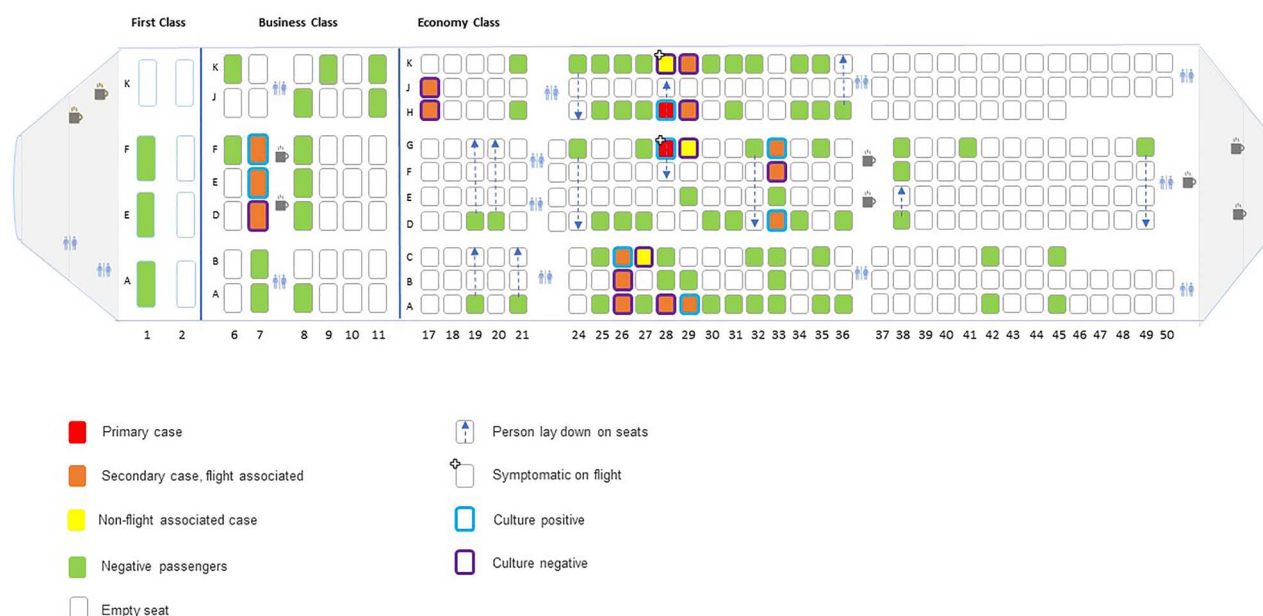


Figure 3. Seating location of passengers on flight from Dubai to Perth, 1 July 2020.

Table 1. Risk of SARS-CoV-2 infection by time spent at Perth airport, mask wearing, seating location and both seating location and mask wearing for passengers on flight from Dubai to Perth, 1 July 2020

	Secondary flight-associated cases	Negative passengers	Relative risk	Risk ratio	95% CI	P value
Time spent at Perth airport	<i>n</i> = 13	<i>n</i> = 58				
Greater than 1 hour	10	25	0.4	3.43	1.03–11.42	0.04
1 hour or less	3	33	0.08			
Mask wearing for passengers interviewed on the flight	<i>n</i> = 13	<i>n</i> = 58				
Not all the time	5	8	0.38	2.79	1.09–7.15	0.05
All the time (including exceptions)	8	50	0.14			
Seating location for all passengers in relation to the index cases	<i>n</i> = 15	<i>n</i> = 75				
≤2 seats away	7	15	0.32	2.71	1.11–6.61	0.05
>2 seats away	8	60	0.12			
Mask wearing for passengers interviewed within two rows of the primary cases on the flight	<i>n</i> = 7	<i>n</i> = 58				
Not all the time	3	4	0.43	6.21	1.74–22.24	0.02
All the time (including exceptions)	4	54	0.07			

confidence interval (CI) 1.11–6.61; $P=0.05$] times greater risk of becoming a secondary case compared to those that were seated more than two rows away (Table 1). Passengers that did not wear a mask all the time on the flight were at 2.79 (95% CI 1.09–7.15; $P=0.05$) times greater risk of becoming a secondary case compared to those that wore a mask all the time. Passengers seated within two rows of the primary cases who did not wear a mask all the time on the flight were at 6.21 (95% CI 1.74–22.24; $P=0.02$) times greater risk of becoming a secondary case compared to those that were seated within two rows of a primary case and wore their mask all the time. Passengers that spent greater than 1 hour at Perth airport were at 3.43 (95% CI 1.03–11.42; $P=0.04$) times greater risk of acquiring SARS-CoV-2 infection compared to those that spent 1 hour or less at the airport.

In multivariate analyses simultaneously controlling for seating proximity, mask use on the flight and time at Perth Airport, being seated within two rows of the primary cases [odds ratio (OR) 7.16; 95% CI 1.66–30.85; $P=0.01$] and spending greater than 1 hour at Perth airport (OR 4.96; 95% CI 1.04–23.60; $P=0.04$) were independently significant at increasing the odds of becoming a secondary case (Table 2).

For all passengers, self-reported increased hand washing, wearing gloves, spending longer than 2 hours at Dubai airport and more than 1 hour on the bus from Perth airport to hotel quarantine were not significantly associated with the risk for becoming a secondary case. For economy class passengers, moving up and down the aisle more than three times or using the bathroom during the flight also did not significantly increase the risk of becoming a secondary case. Mask wearing behaviour

Table 2. Logistic regression of significant risk variables related to odds of SARS-CoV-2 infection

	Odds ratio	95% CI	P value
≤2 seats away from primary cases	7.16	1.66–30.85	0.01
Greater than 1 hour at Perth airport	4.96	1.04–23.60	0.04
Not wearing a mask all of the time on the flight from Dubai to Perth	2.98	0.64–13.84	0.16

outside of the plane environment was not significantly associated with a reduced risk of becoming a secondary case (Appendix 2).

Discussion

Our investigation identified substantial flight-associated transmission of SARS-CoV-2, similar to the 16.6% secondary attack rate reported from a systematic review of household transmission.¹¹ As has been reported previously, being seated near persons who are infectious during a flight increased the risk of passengers experiencing secondary infection.^{3,12} This supports that the two-row contact tracing method is able to identify most, but not all, of the secondary flight-associated SARS-CoV-2 infections.

Furthermore, our investigation found that spending more than 1 hour in disembarkation procedures at the arrival airport independently increased the odds of becoming a travel-associated secondary case. The implication that SARS-CoV-2 transmission occurred outside the cabin environment is further supported by the fact that the three secondary cases in business class had no contact with the primary cases seated in economy during the flight, but all three spent 2 hours unsegregated from economy class passengers while transiting through checkpoints and waiting for paperwork to be processed at Perth Airport. In contrast, the check-in, boarding and deplaning processes for this flight would appear to be less likely as the site of exposure for secondary cases travelling in business class because they used separate service counters and entrances to board and deplane from those in economy. In this investigation, other possible opportunities for exposure between passengers in different travel classes included the bus ride from Perth Airport to the quarantine hotel and time spent before boarding at the departure airport, but neither of these settings was implicated as a significant predictor of increased risk in epidemiologic analyses.

Mask wearing on the flight offered some protection against secondary infection, with even greater protection observed for passengers seated within two rows of the primary cases. Although most passengers removed their masks to eat and drink, there appear to be demonstrated benefits in maintaining a high level of mask wearing in-flight, which has also been supported by other studies.^{1,4,6,7,12,13–15}

The aviation industry has been vocal about the safety of commercial air travel during the COVID-19 pandemic,^{16–18} adopting increased cleaning processes, ensuring that flight crew are wearing personal protective equipment, and providing this equipment to passengers. All of the airline's aircraft are fitted out with HEPA filters,¹⁹ which remove nearly 100% of harmful contaminants. However, airflow dynamics on aircraft are influenced by many factors such as passenger and flight-crew movements, occupancy density, direction of air vents and cabin geometry.^{20–22} This

outbreak demonstrated a high attack rate despite the functioning HEPA system and enhanced personal measures adopted by flight crew and many passengers, indicating that the effect of cabin airflow on transmission dynamics of SARS-CoV-2 on aircraft needs further investigation.

This study highlights the importance of a combined epidemiological and genomics approach in conducting a comprehensive outbreak investigation, and challenges in matching sequences to their geographic origin. No international matches were found for the sequences within this genomic cluster, consistent with the sequence originating in a region with limited genomic surveillance for SARS-CoV-2. However, our findings are supported by the high prevalence of B.1.480 lineage sequences reported from Ethiopia, where the primary cases commenced their travel.

There were several limitations with our investigation. First, the 79% response rate to the passenger interviews is reasonably high, but the non-response of some participants may still have introduced bias. Recall bias may also have occurred if persons who became ill after the flight differed in their recollection of events or behaviours. Second, we were unable to conduct more detailed analyses of some potential risk factors, such as hand hygiene practices, or possible differences between children and adults, due to small numbers, so further study may be beneficial. Third, some questions in the passenger interviews allowed for free-text responses and this complicated data aggregation for statistical analysis. Fourth, while there were no subsequent reported infections among the flight crew by the airline, they did not undergo mandatory quarantine and were excluded as participants in this study.

Conclusion

With the emergence of more transmissible SARS-CoV-2 variants, for example Omicron,^{23–26} it is crucial to understand and mitigate potential risk exposures associated with all stages of air travel. While vaccinations may reduce the risk of SARS-CoV-2 transmission in the future, the potential for vaccine breakthrough and re-infection makes it is likely that non-pharmaceutical interventions will need to be continued, and our study demonstrated that conscientious mask wearing during travel reduced the risk of acquiring infection.^{24,27,28} In addition, most previous studies of flight-associated disease transmission have focused on the potential risk of exposure while on the plane. Importantly, our investigation identified that there may be risks associated with air travel occurring outside the cabin environment. Increased awareness among the airline industry, regulators, airport authorities and passengers of the opportunity to reduce exposures to infectious pathogens at all stages of a journey could enable safer air travel going forward.

Contributors

P.V.E. and F.V. conceived and designed the study. S.N. collected the data. S.N., F.V. and N.M.P. analysed the epidemiological data. C.T.S., A.L. and D.W.S. undertook formal phylogenetic analysis. C.T.S. and A.L. curated the data. S.N. and C.T.S. wrote the manuscript and all authors reviewed and edited the manuscript.

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Supplementary data

Supplementary data are available at *JTM* online

Conflict of interest

The authors have declared no conflicts of interest.

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