SARS-CoV-2 Seroprevalence in a Cohort of International Travellers Returning to Rural Australia: Enablers and Barriers to Containment of COVID-19

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Abstract

Objective: To describe the effectiveness of the public health response to COVID-19 in our local region by documenting detection of SARS-CoV-2 infection by nucleic acid testing (NAT) positivity and seroprevalence.

Methods: In this prospective study (ACTRN12620000487910), symptomatic adult international travellers returning to regional Australia in March 2020 underwent SARS-CoV-2 NAT and SARS-CoV-2-specific serology.

Results: Ninety-nine eligible participants were included. Nine participants had laboratory confirmed SARS-CoV-2, all returning between 16–20 March 2020. Eight (89%) had a positive NAT and seven (78%) had a positive serology test. The majority returned from New Zealand. Participants most frequently presented with cough (100%), headache (66.7%) and sore throat (44.4%). No community cases were detected from 1 March to 30 June 2020.

Conclusions: The study cohort of international travellers returning to regional Australia in March 2020 returned eight positive SARS-CoV-2 NAT results over a five-day window. Serology identified one additional case and was negative in two cases who were PCR positive. Longitudinal data confirmed an absence of local community transmission to 30 June 2020.

Implications for public health: A combination of local, national and environmental factors were necessary to prevent the establishment of community transmission in our local region.

Key words: Australia, COVID-19, SARS-CoV-2, serology, epidemiology

Introduction

SARS-CoV-2, the causative agent of COVID-19, was first recognised in Wuhan, Hubei province, China in December 2019 before spreading globally.[1] In Australia, initial cases of COVID-19 stemmed almost exclusively from international travellers returning from high prevalence countries.[2] The first confirmed coronavirus case was identified on the 25 January 2020, with the first 15 confirmed cases arriving from Hubei Province.[3] In February, subsequent cases were identified among the 'Diamond Princess' cruise ship passengers repatriated on a flight from Japan and by mid-March, positive COVID-19 cases were confirmed among travellers from Iran, Italy, the United Kingdom and the United States of America. By the end of March, there were 4,159 confirmed cases of COVID-19 in Australia.[4]

The National Cabinet of Australia responded by implementing universal precautionary self-isolation requirement on all international arrivals, effective from the 16 March 2020 and closing its borders to all non-citizens and non-residents from 9pm AEDT Friday, 20 March

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2020.[5] By 29 March, all travellers arriving in Australia were required to undertake mandatory 14-day self-isolation at designated facilities.[6]

As case numbers increased and the scope of the threat became apparent, health care facilities in both rural and metropolitan Australia grappled with the best approach to contain the emerging pandemic. In this paper, we describe our experience at Albury Wodonga Health, with a focus on the use of nucleic acid testing (NAT) and serological testing. Albury Wodonga Health is a cross-border regional hospital servicing rural southern New South Wales and northeast Victoria with a population catchment area of over 280,000 people and with approximately 70,000 emergency department presentations annually.

Methods

A combined retrospective and prospective study was conducted with approval from the Albury Wodonga Human Research Ethics Committee (ERM/63202). All international travellers 18 years of age and over who had returned to Australia between 1 January and 31 March and who received SARS-CoV-2 nucleic acid testing (NAT) through Albury Wodonga Health Service were approached for inclusion in this study. People were excluded from the study if they were younger than 18 years of age, died prior to the collection of sera for SARS-CoV-2 serology, did not travel internationally or did not receive SARS-CoV-2 NAT.

Case acquisition

Albury Wodonga Health Service established a drive-through COVID-19 screening clinic on 5 March 2020. Public health messaging encouraged community residents who were concerned they may have COVID-19 to contact the health service through a centralised local COVID-19 phone hotline. Calls were screened by nursing staff with a standardised questionnaire that included clinical data on current symptoms and epidemiological data on overseas travel. All people with symptoms consistent with COVID-19 were offered SARS-CoV-2 NAT and those had returned from overseas to Australia between 1 January and 31 March inclusive were approached for inclusion in our study.

Swabbing technique

Participants received pharyngeal, anterior nares and nasopharyngeal brushings using a single flocked swab. The swabs were placed in viral transport medium and delivered via road transport within four hours to Melbourne for further processing.

SARS-CoV-2 qRT-PCR testing

The samples were processed on LightCycler® 480 in line with the manufacturer's protocols. All NAT positive results were then forwarded on to the Victorian Infectious Disease Reference Laboratory (VIDRL) for confirmatory testing.

Serology testing

Sera for SARS-CoV-2-specific serology was collected a minimum of 14 days after the initial symptom onset. Serology was performed at NSW Health Pathology using an in-house immunofluorescence assay (IFA) for SARS-CoV-2-specific IgG, IgA and IgM, as well as total antibody confirmation by virus microneutralisation.[7] The date of collection and the immunofluorescent titre for each antibody class were recorded.

Data analysis

Data were extracted from electronic medical records and patient interviews and entered into a purpose-built database in ClinCapture Captivate Electronic Data Capture (EDC). The following data were collected for all episodes: patient demographics (age, gender, occupation and comorbidities), travel history (duration of travel, date of return to Australia, countries visited and travel on a cruise), illness details (date of symptom onset and symptomatology), influenza vaccination and the date and result of SARS-CoV-2 NAT. The Charlson comorbidity index was used to categorise participants according to the severity of comorbidities.[8]

Categorical variables were reported as counts and percentages and continuous variables as mean and standard deviation. We compared categorical variables applying Fisher's exact tests and for continuous variables Mann-Whitney U tests were performed. A p-values <0.05 was considered significant.

The analysis was performed using Stata/IC 15.1 (StataCorp. 2017. *Stata Statistical Software: Release 15*. College Station, TX: StataCorp LLC) and R (version 3.6.2 R Project for Statistical Computing) within RStudio.

Results

Ninety-nine out of 256 participants were included in this study according to the inclusion and exclusion criteria. A flow chart of study disposition is presented in **Figure 1**, divided between those with negative or positive SARS-CoV-2 NAT as subjects were aware of their NAT result when giving consent to participate in the study.

Eight participants had a positive SARS-CoV-2 NAT result. Out of the remaining 91 participants who had negative SARS-CoV-2 NAT, one additional case was detected through serological testing. This resulted in a total of nine participants who were classified as positive cases for the purposes of further analysis. Eight of the nine positive cases (89%) had a positive NAT and seven out of nine (78%) had a positive serology test (Table A1).

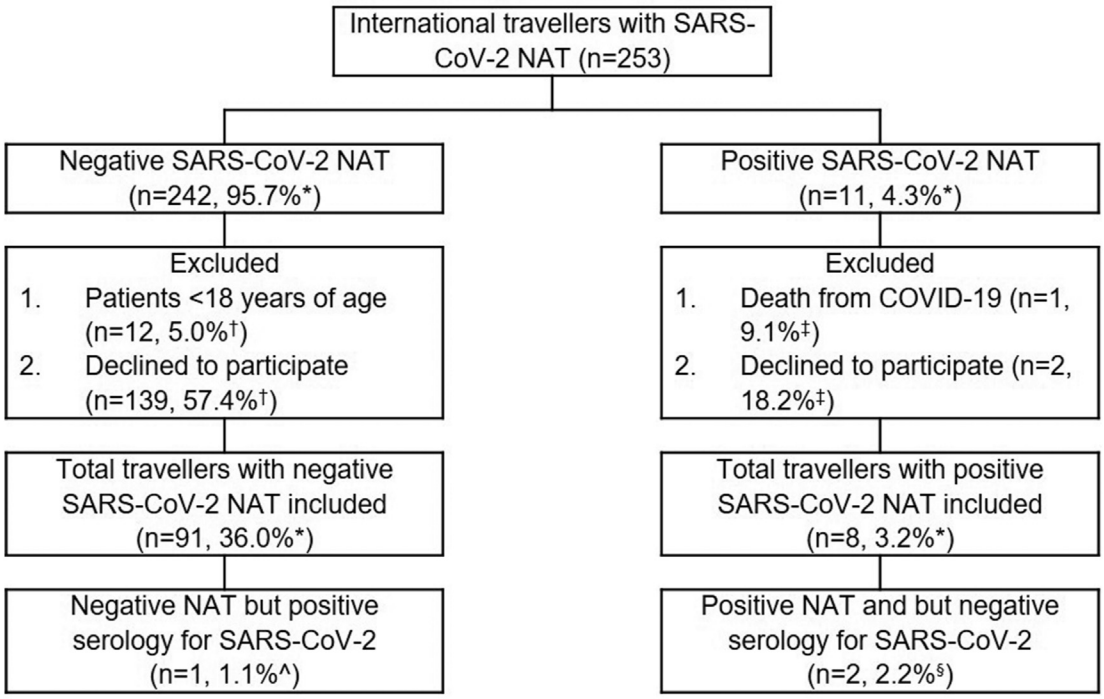
Table 1 provides patient characteristics and symptoms comparing participants with negative result (n=90) to those with a positive result (n=9) for SARS-CoV-2. The average age of participants was 50.7 years of age, with significant differences between the SARS-CoV-2 positive and negative cases. Participants with positive results had an average age of 58.8 years, with a range of 21 to 74 years, while those with negative results were younger on average at 49.9 years with a range from 18 to 89 years (p=0.049). Positive cases were also significantly more likely to have a higher Charlson Comorbidity Index.

There was no statistical difference in gender, history of travelling on a cruise ship or smoking between the positive and negative cases. Overall, 20% of participants had travelled on a cruise ship, with no statistical difference in the age of those that went on a cruise and those who did not. For the positive cases, we observed that 44.4% returned from a cruise, all travelling to New Zealand, compared to 16.7% in those who returned a negative result (Table A2).

The three most common presenting symptoms were cough, sore throat and headache. We observed statistical difference between the groups only for cough.

Figure 2 and Figure A1 show the chronology of participants returning from international travel, symptom onset, NAT and serologic testing with a breakdown provided for positive and negative cases,

Figure 1: Study methodology. *Percentage of cases from all international travellers (n=253). ^Percentage of the sample with a negative SARS-CoV-2 NAT (n=242). †Percentage of the sample with a negative SARS-CoV-2 NAT. ‡Percentage of the sample with a positive SARS-CoV-2 NAT (n=11). ^Percentage of the sample included with a negative SARS-CoV-2 NAT (n=91). §Percentage of the sample included with a positive SARS-CoV-2 NAT (n=91).



respectively. Positive cases all returned to regional Australia between 16–19 March 2020.

The mean time from symptom onset to NAT was three days for positive cases (range 0-9 days) and five days (range 0-23 days) for negative cases. The mean time from symptom onset to serological testing was 27 days for positive cases and 58 days for negative cases.

Participants 1 through 6 had both a positive NAT and positive serology. Symptoms developed an average of three days after returning to Australia (range days 1-6). Serological testing was performed from day 22 through to day 35 following symptom onset.

Patient 7 returned from New Zealand and described cough, sore throat and a headache. They were tested on day two of symptom onset with a cycle threshold value of 14.45 and confirmatory testing was positive. Serological testing was performed on day 18 following symptom onset and was reported as negative.

Patient 8 returned from the United States of America with symptoms consisting of cough, sore throat and headache and myalgia five days prior to return. NAT was performed on day nine of symptoms and returned cycle threshold values of 35.11 on initial testing and 35.31 following re-extraction. Further testing using the same sample at the reference laboratory was negative. Serology was performed on day 45 following symptom onset and was reported as negative.

Patient 9 developed symptoms 18 days following return to Australia from travel to New Zealand on board the 'Celebrity Solstice'. She had a negative NAT but positive serology. Her symptoms consisted of a cough, sore throat and headache. Serological testing was performed day 27 and day 71 following symptom onset and demonstrated a SARS-CoV-2-specific IgG of 20 falling to 10 on repeat testing, with an IgM and an IgA titre <10 on both occasions.

Patient 10 was treated clinically as a presumptive positive despite both negative NAT and negative serology. They were a close household contact of Patient 5 and had both travelled on the 'Ruby Princess' cruise ship. Patient 10 had symptoms of headache and diarrhoea. NAT on day three following symptom onset was reported as negative. Serology was taken on day 21 following symptom onset and was also reported as negative.

Figure 3 explores the country of travel with a breakdown of negative and positive cases. The majority of international travellers returned from New Zealand (n=27), which also generated the majority of COVID-19 cases (n=7). Of those travelling to New Zealand, four confirmed cases and one probable cases (Patient 10) also travelled on a cruise (Table A2). The USA was the next most common country of travel (n=14) with a single confirmed case. The remaining case was detected in a traveller returning from Bali in Indonesia.

During the period from 1 March through to 30 June 2020, three people with coronavirus were admitted to Albury Wodonga Health Service, resulting in a combined total of 21 bed days. All three people were international travellers and had been previously identified as infected with SARS-CoV-2 through the drive-through swabbing clinic. They bypassed the emergency department and were admitted to single rooms. Negative pressure facilities were unavailable. N-95 masks, gowns and gloves wore worn. One patient required intubation.

In the period from 1 March to 30 June 2020, a total of 5,316 SARS-CoV-2 NAT were performed in the drive through clinic for symptomatic community members, with 4,721 of those tests performed from 1 April to 30 June 2020. An additional 957 SARS-CoV-2 NAT were

Table 1: Patient characteristics and symptoms comparing participants with negative result (n=90) to those with a positive result (n=9) for SARS-CoV-2.

Patient Characteristics		SARS-CoV-2 Negative	SARS-CoV-2 Positive	Total	p
Age	Mean (SD)	49.9 (16.2)	58.8 (16.2)	50.7 (16.4)	0.049
Gender	Female	48 (53.3)	5 (55.6)	53 (53.5)	0.59
	Male	42 (46.7)	4 (44.4)	46 (46.5)	
Charlson Comorbidity Index	0	35 (38.9)	2 (2.22)	37 (37.4)	0.021
	1	27 (30.0)	0 (0)	27 (27.3)	
	2	21 (23.3)	4 (44.4)	25 (25.2)	
	3	5 (5.6)	3 (33.3)	8 (8.1)	
	4	1 (1.1)	0 (0)	1 (1.0)	
	6	1 (1.1)	0 (0)	1 (1.0)	
Cruise	No	75 (83.3)	5 (55.6)	80 (80.8)	0.066
	Yes	15 (16.7)	4 (44.4)	19 (19.2)	
Smoking history	No	51 (56.7)	4 (44.4)	55 (55.6)	0.505
	Yes	39 (43.3)	5 (55.6)	44 (44.4)	
Symptom	Cough	61 (67.8)	9 (100.0)	70 (70.7)	0.038
	Sore Throat	58 (64.4)	4 (44.4)	62 (62.6)	0.204
	Headache	38 (42.2)	6 (66.7)	44 (44.4)	0.146
	Chills	23 (25.6)	0 (0.0)	23 (23.2)	0.082
	Muscle aches	22 (24.4)	2 (22.2)	24 (24.2)	0.623
	Fever	21 (23.3)	2 (22.2)	23 (23.2)	0.652
	Coryzal*	17 (18.9)	0 (0.0)	17 (17.2)	0.169
	Diarrhoea	14 (15.6)	3 (33.3)	17 (17.2)	0.182
	Dyspnoea	13 (14.4)	2 (22.2)	15 (15.2)	0.41
	Abdominal Pain	8 (8.9)	1 (11.1)	9 (9.1)	0.091
	Tiredness/Fatigue	8 (8.9)	1 (11.1)	9 (9.1)	0.592
	Vomiting	1 (1.1)	1 (11.1)	2 (2.0)	0.174
	No Symptoms	5 (5.6)	0 (0.0)	5 (5.1)	0.614
	Other†	13 (14.4)	0 (0.0)	13 (13.1)	0.266

*Coryzal symptoms were runny nose, sneezing and nasal congestion. †Other symptoms were chest tightness, dizziness, fibromyalgia, heavy in head, itchy throat, light headed, nausea, sinus pain, sore eyes, swollen glands

performed on people presenting directly to Albury Wodonga Health, including both the emergency department and inpatient wards, from 1 March through to 30 June 2020 with 889 of those performed from 1 April to 30 June 2020. No additional cases were detected.

Discussion

This study captures a unique time period in the evolution of a pandemic resulting from a novel infection. While serology and NAT were available during the study period, rapid antigen testing, effective treatment and vaccination were not tools available for public health intervention. Our data therefore provide valuable insights to inform both government and regional health authorities on the importance of disease surveillance of international travellers early in a pandemic.

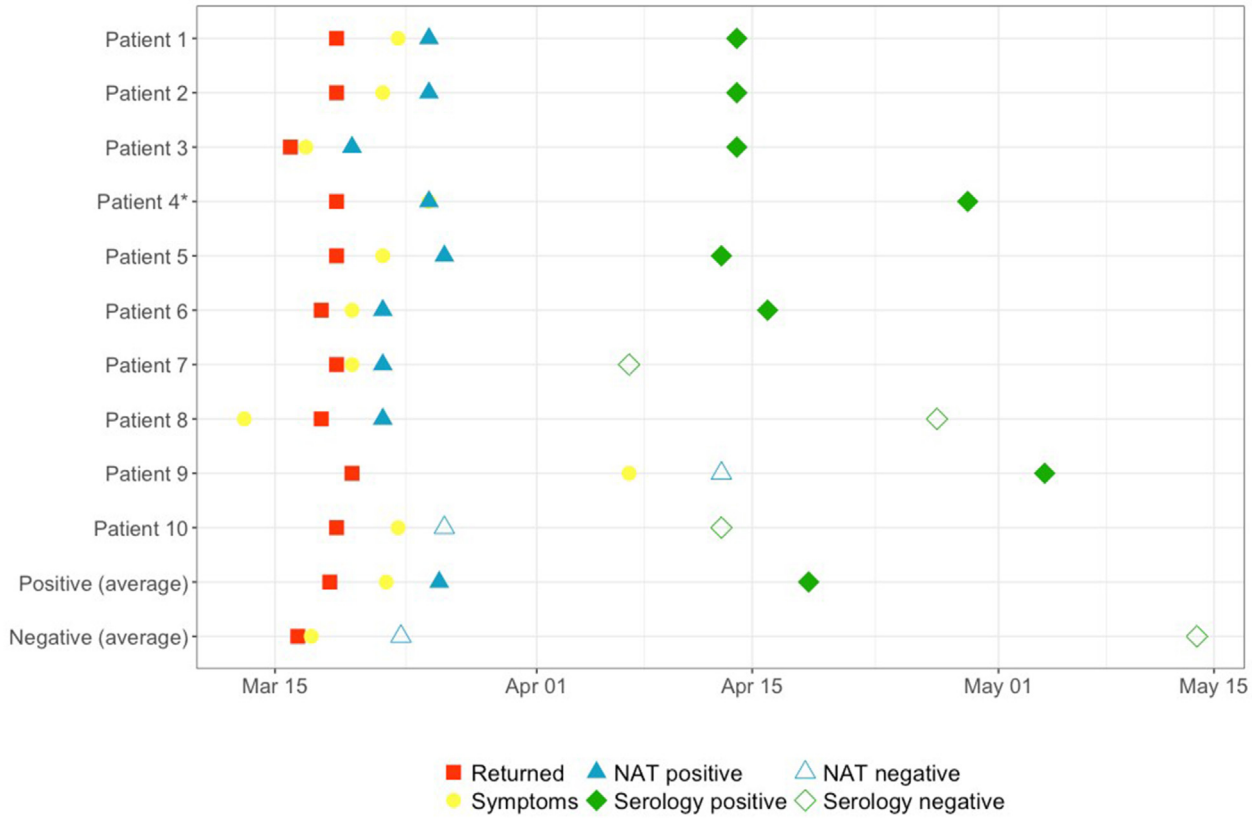
The sustained and prolonged absence of COVID-19 cases in our region despite ongoing rigorous testing strongly suggests an absence of local community transmission. It is recognised that the sensitivity of the testing regimen within a given context is an important measure.[9] While we do not know if there were undetected cases in our community who did not transmit the virus, the longitudinal data through to 30 June 2020 provides evidence that our screening program captured all relevant cases who may have otherwise continued the transmission cycle. Apart from viral transmission dynamics, there are likely to have been multiple factors that contributed to containment of cases to those with established infection acquired during international travel and the prevention of local community transmission.

NAT test characteristics

NAT on nasopharyngeal swabs has been widely adopted in Australia as the standard diagnostic test for SARS-CoV-2 infection. In idealised conditions, these assays are reported to be highly sensitive and specific for SARS-CoV-2.[10] The clinical performance in real-world conditions may differ substantially though and relies on the amount and quality of RNA in the collected samples.[11] Studies estimating test performance characteristics have imperfect design and statistical methods and are hampered by the lack a gold-standard comparator. As a result, the estimated sensitivity and specificity varies widely in studies reported, ranging from 40% through to 100%.[12] Sensitivity is also dependent on the site of sampling[13] and the time point at which the sample is taken during the course of the illness.[14] Bronchoalveolar lavage fluid is reported to give the best yield, followed by sputum and nasopharyngeal specimens, with poorer sensitivity from oropharyngeal and stool samples.[15]

Serology testing is a retrospective method for detecting COVID-19 infection and complements SARS-CoV-2 NAT. Serological testing can confirm SARS-CoV-2 infection by identifying seroconversion of SARS-CoV-2-specific IgG, IgA and IgM antibodies.[13] The time to seroconversion makes serological testing suboptimal for diagnosing acute infection, but it remains an important tool for calculating

Figure 2: Chronology of the date return from international travel to Australia, date of symptom onset, date of NAT and date of serology testing with a breakdown for all laboratory confirmed and clinically suspected cases. *Patient 4 developed symptoms on the same day as the SARS-CoV-2 NAT was performed.



seroprevalence, measuring vaccine response, testing for previous infection and for individuals presenting late in their clinical course, where NAT may yield negative or false-positive results.[16,17] In the validation study of the assay used in this study, the mean time to develop antibodies was 10.2 days with a range of 5.8-14.4 days.[7] Utilising 126 individuals who were NAT positive as the reference standard, 91.3% of cases developed antibodies. In our sample, and excluding Patient 10, the calculated seroconversion rate was 75%. A limitation of our study is that participants with a negative NAT were less likely to proceed to serology (91/242 or 37.6%) compared to those with a positive NAT (8/11 or 72.7%).

One patient in our cohort (Patient 9) had a negative NAT but serologic evidence of prior infection with SARS-CoV-2. While the patient's symptoms were consistent with COVID-19, the symptom onset was 18 days after returning to Australia and outside of the usual incubation period of up to 14 days. This suggests that this patient had in fact had COVID-19 prior to the time of SARS-CoV-2 NAT testing and that this result was a true negative.

Patient 10 was both a returned international traveller and a household contact of a confirmed case who was treated clinically as a presumptive positive as his symptoms met case definition for COVID-19. Both SARS-CoV-2 NAT and serology were negative in this patient. Despite the high pre-test probability, the possibility remains that both tests are true negatives. Assuming the latter and taking into consideration the absence of community transmission, the estimated sensitivity of NAT on oro-naso-pharyngeal/anterior nares swabs in our cohort was 100%.

Timing of Government initiatives

The low seroprevalence (1/91 or 1.1%) in NAT negative participants suggests a low exposure rate to SARS-CoV-2 in returned travellers prior to 16 March. NAT positive cases not only returned to rural Australia but were also were confirmed as positive in an extremely narrow time window. This comprised only five days from 16 to 20 March and coincides precisely with the orders to self-quarantine and the international border closure, respectively. This suggests that the timing of these government initiatives was a crucial determinant in preventing community transmission within Australia at this time.

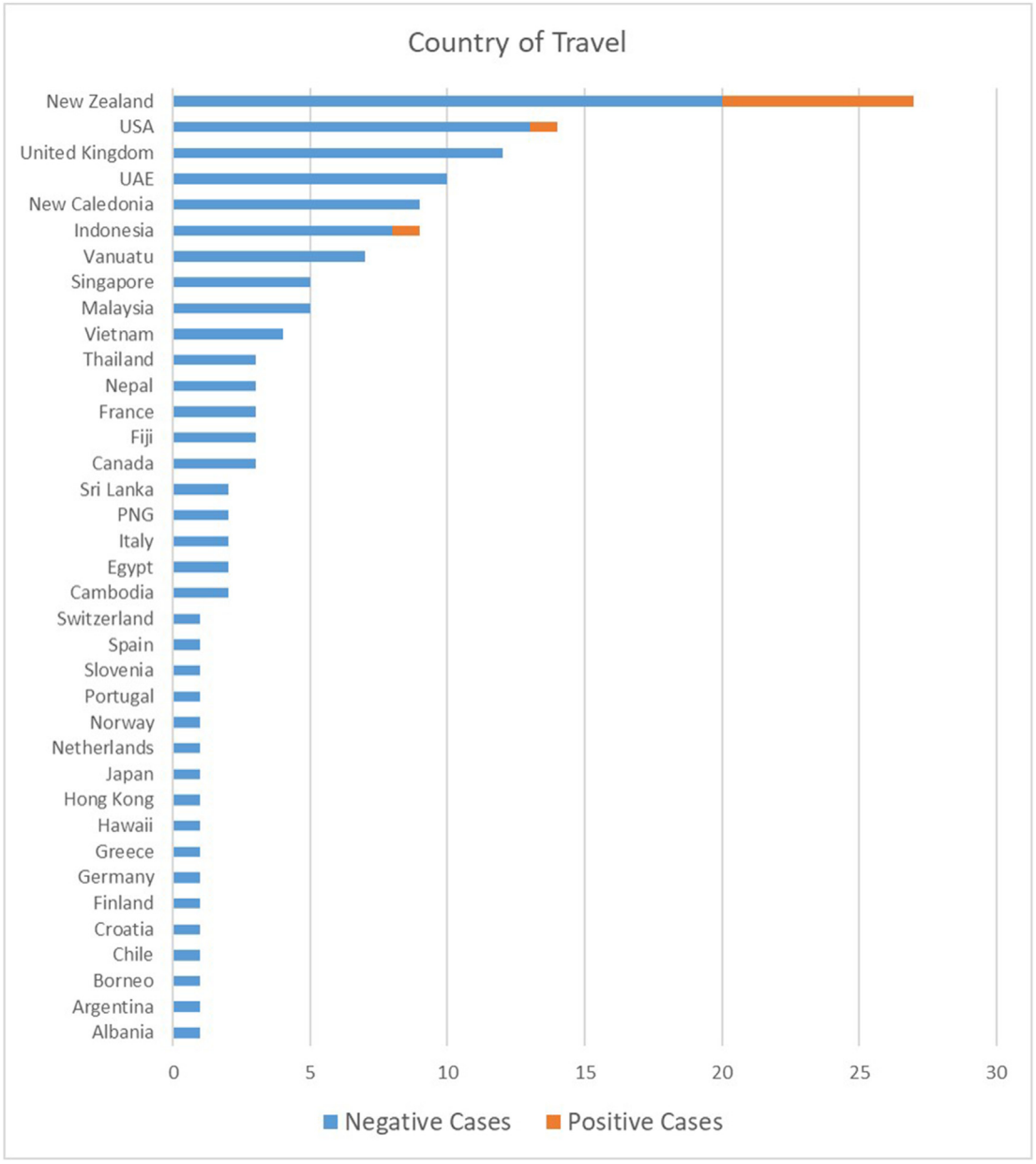
Community response and engagement

Messaging around the clear epidemiological risk of overseas travel facilitated detection of positive cases. Community awareness and engagement was excellent, as evidenced by the total number of international travellers presenting for testing.

Isolation of household contacts

Specific to our program, secondary household contacts of symptomatic international travellers were requested to voluntarily isolate until the results of the SARS-CoV-2 NAT were known. This policy was later more widely adopted as a public health measure in Victoria in order to prevent ongoing transmission from secondary contacts while the results of the primary case were pending.

Figure 3: Country of travel.



External testing location

It was recognised early that people presenting to the emergency department or general practice for testing posed significant risk of transmission of COVID-19 to health-care workers and other patients. This realisation resulted in the rapid initiation of an off-site drive-through screening clinic. All confirmed COVID-19 patients were first detected via NAT in the drive-through clinic. Those patients who subsequently required admission bypassed the emergency department and were admitted directly to single rooms on the ward.

Infection control measures in hospital

Patients were cared for in a single room with contact and droplet precautions along with N95 masks. While evidence of airborne transmission for SARS-CoV-2 was scant in March 2020, airborne spread with SARS-CoV-1 was suggested from the Amoy Gardens outbreak.[18] Subsequent published data specific to SARS-CoV-2 confirmed airborne transmission.[19,20] The probability of airborne transmission is dependent on a number of factors including ventilation and air filtration, size of the enclosed space, breathing rate, respiratory activity, use of face masks, procedures undertaken and infectiousness of the respiratory aerosols.[21,22] Neither a dedicated ward nor negative pressure rooms were available to care for the inpatients infected with SARS-CoV-2. Containment in our region must therefore be viewed as fortunate with significant risk if the same infection control measures were to be used in the event of further cases.

External factors

Seasonal and environmental factors are likely to have had their part to play in the success of our program. March follows the end of the Australian summer where a larger proportion of time is spent

outdoors in the fresh air and when vitamin D levels are likely to be at the peak. Replete Vitamin D levels are correlated not only with decreased severity of disease but also with decreased infection rates.[23] Likewise, outdoor air is recognised to contain viricidal properties and limit the spread of contagion.[24] Regional Australia differs substantially from metropolitan locations in regard to population density, high-rise buildings, availability and utilisation of public transport and dining preferences.

Conclusion

Our study documents that in a cohort of international travellers returning to regional Australia in March 2020, positive cases were detected in only in a very discrete time window. Serology identified one unrecognised case who was likely to have had illness at an earlier time point and was negative in two cases who were PCR positive. Longitudinal data confirmed an absence of local community transmission through to 30 June 2020. The prevention of secondary transmission within our region was enabled by a combination of local and national public health initiatives including a local screening program utilising SARS-CoV-2 NAT, isolation of symptomatic returned travellers and their household contacts, and closure of international borders.

Funding

No funding was recieved for this study.

Ethics

This study was conducted with approval from the Albury Wodonga Human Research Ethics Committee (ERM/63202).

Conflict of interest

None.

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Appendix A Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.anzjph.2022.100003.