Detection of influenza in managed quarantine in Australia and the estimated risk of importation

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Abstract

Background

Influenza circulated at historically-low levels during 2020 and 2021 due to COVID-19 pandemic travel restrictions. In Australia, international arrivals to Australia were required to undertake 14 days hotel quarantine to limit new introduction of SARS-CoV-2 virus.

Methods

We used routine testing data for travellers arriving on repatriation flights to Darwin, Australia from 3 January to 11 October 2021 to identify importations of influenza virus into Australia and used this information to estimate the risk of a case exiting quarantine while still infectious. Influenza-positive samples were sequenced and cases were followed-up to identify transmission clusters. Data on the number of cases and total passengers was used to infer the risk of influenza cases existing quarantine while infectious.

Results

Despite very low circulation of influenza globally, 42 cases were identified among 15,026 returned travellers, of which 30 were A(H3N2), two were A(H1N1)pdm09 and 10 were B/Victoria. Virus sequencing data identified potential in-flight transmission, as well as independent infections prior to travel. Under the quarantine strategy in place at the time, the probability that these cases could initiate influenza outbreaks in Australia neared 0. However, this probability rose as quarantine requirements relaxed.

Conclusions

Detection of influenza virus infections in repatriated travellers provided a source of influenza viruses otherwise unavailable and enabled development of the A(H3N2) vaccine seed viruses included in the 2022 Southern Hemisphere influenza vaccine. Failing to test quarantined returned travellers for influenza, represents a missed opportunity for enhanced surveillance to better inform public health preparedness.

Introduction

At the beginning of the coronavirus disease 2019 (COVID-19) pandemic, a number of countries enforced travel restrictions to limit introductions of the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) (1). The Australian Government closed its borders to non-residents on 20 March 2020 and required returning travellers to undergo 14 days quarantine in managed hotels from 28 March 2020 (2). This policy had a dramatic effect on limiting introductions of SARS-CoV-2 viruses, and in tandem with non-pharmaceutical interventions (NPIs), meant that most Australian jurisdictions had no or little local transmission of SARS-CoV-2 by around June 2020 (3).

These measures also prevented introductions and circulation of other respiratory viruses, most notably influenza (4). In Australia, as well as globally, circulation of influenza in 2020 and 2021 was at historical lows (4, 5). However, the virus continued to be detected in isolated pockets around the world, notably in tropical regions of Asia and West Africa (6). Here, we present data collected from testing of all returned travellers arriving at a quarantine facility in Darwin, Australia. This provided a unique opportunity to study the rate at which travellers arriving in Australia tested positive for influenza, information which informed expectations about the likelihood of travellers initiating an epidemic as travel restrictions relaxed. Moreover, it augmented influenza virological surveillance and enabled development of influenza candidate vaccine viruses that might otherwise have been unavailable.

Methods

The Australian Federal Government in partnership with QANTAS, operated repatriation flights in 2020 and 2021, many of which arrived in Darwin. Passengers were required to return both a

negative COVID-19 Polymerase Chain Reaction (PCR) test and a negative Rapid Antigen test before boarding, be asymptomatic and wear a face mask for the duration of the flight. Upon arrival, travellers were transferred to a large low-rise quarantine facility, located at nearby Howard Springs, for a minimum of 14 days quarantine. Nasal and throat samples were taken on-arrival, 7 and 12 days after arrival, and when indicated due to symptoms or being a close contact of a SARS-CoV-2 case. Samples were tested at Territory Pathology for Influenza A&B, SARS-CoV-2 and Respiratory Syncytial Virus.

Cases testing positive for influenza were contacted by the Northern Territory Centre for Disease Control (NT-CDC) to identify family and travelling groups and confirm flight information and port of origin. To understand the epidemic situation in the country of origin, influenza data were downloaded from the World Health Organization's (WHO) FluNet platform (https://www.who.int/tools/flunet), while COVID-19 epidemic data were downloaded from the WHO Coronavirus (COVID-19) Dashboard (https://covid19.who.int/data).

Virus characterisation

Influenza-positive samples were forwarded to the WHO Collaborating Centre for Reference and Research on Influenza in Melbourne for antigenic and genetic characterization. Viruses were first grown in MDCK cells to obtain virus isolates. Isolates were tested in haemagglutination inhibition assay to assess their similarity to the 2021 southern hemisphere vaccine viruses; i.e. A/Victoria/2570/2019 (H1N1pdm09), A/Cambodia/e0826360/2020 (H3N2), B/Washington/02/2019 (B/Victoria lineage). The haemagglutinin gene of virus isolates or the original specimen if an isolate was unavailable was sequenced using Sanger or Illumina iSeq as previously described (7). Phylogenetic analysis was performed using the Augur pipeline (8),

which uses IQTree (9) for constructing and bootstrapping (-B 1000 -alrt 1000) the phylogenetic tree (model: GTR) and finally visualised using ggtree (10). Sequences were deposited in GISAID, accession and acknowledgements are in Supp Table 2.

Risk of influenza escape from quarantine

Given the short incubation period, infectious period and serial interval of influenza (11) it is unlikely that cases detected in quarantine would still be infectious on day 14. To assess this risk under various quarantine scenarios, the observed detections were used to inform a Bayesian framework previously established to assess the risk of SARS-CoV-2 escaping quarantine (12). The model considered disease prevalence, travel volume, control strategies and their effectiveness, and the natural history of disease to estimate the influenza importation risk.

Disease prevalence was calculated based on the number of influenza detections for each port of origin among the total number of passengers arrived from that port, provided by the NT-CDC. Based on quarantine requirements in place at the time, the framework assumed that all passengers received an on-arrival SARS-CoV-2 test, with reflexive testing for influenza if SARS-CoV-2-negative, and received their test results prior to exit. Five different quarantine scenarios were explored: 1) no quarantine; 2) 7 days quarantine with no testing; 3) 7 days quarantine with testing on day 5; 4) 14 days quarantine with no testing; and 5) 14 days quarantine with testing on day 12.

Model assumptions were updated from the previous SARS-CoV-2 model using published estimates for influenza. We assumed exposure time before arrival to be no more than 3 days (13). Viral load was set to peak 2 days after exposure (range 1-4) (14). The infectious period followed a gamma distribution that assumed infectiousness peaked with peak viral load, irrespective of

symptoms (11). One-third of cases were assumed to be asymptomatic (15). Test specificity was assumed to be 1 while sensitivity varied according to the day of the test, peaking with peak viral load and halving if the case was asymptomatic (16).

Posterior distributions from 2,000 simulations were calculated. Additional information about the model is available in (12).

Results

Between 03 January and 14 October 2021 89 repatriation flights arrived in Darwin carrying approximately 15,026 passengers. The most common port of origin was New Delhi (n=34 flights; Supplementary Table 1). During this period, 42 travellers tested positive for influenza, 41 from India and one from Pakistan (Supplementary Table 1, Figure 1a). Given the predominance of cases arriving from India, the remainder of the Results focuses on arrivals from India, only.

Thirty cases were influenza A(H3N2), two were A(H1N1)pdm09 and 10 were B/Victoria lineage. The percentage of passengers testing positive for influenza ranged from 0 to 3.7% (Figure 1b). Based on WHO data, detections from India initially occurred as the country was dealing with a surge in SARS-CoV-2 (Delta) cases. India was reporting very few influenza cases at that time (Figure 1c), suggesting that a testing paradigm that only tests when epidemic activity is known to occur in the port of origin would fail to detect cases.

Viruses recovered from passengers on the same flight were not necessarily a single subtype or lineage. On one flight, both A(H1N1)pdm09 and A(H3N2) viruses were detected amongst passengers, and on two flights both A(H3N2) and B/Victoria viruses were detected (Figure 2). Flunet data also suggested circulation of these three viruses in India during the study period (Figure 1c).

Virus characterisation

Twenty-one A(H3N2) viruses were sequenced and all fell in the haemagglutinin (HA) based genetic group 3C.2a1b.2a.2, which represented the dominant genetic clade for A(H3N2) viruses during 2021 (Figure 3). These viruses were genetically distinct from the vaccine virus A/Cambodia/e0826360/2020, which falls in the 3C.2a1b.2a.1 genetic group. This was reflected in HI assay with all isolates low reacting to the vaccine virus (data not shown). On flights with multiple A(H3N2) cases, genetically-similar viruses were detected among both families and unrelated lone travellers on the same flight (e.g. IND38, IND69 in Figure 3), suggesting possible in-flight or in-transit transmission. Less-closely related viruses were also recovered from passengers on the same flight (e.g. IND70 in Figure 3), suggesting independent infections prior to boarding.

One of two A(H1N1)pdm09 viruses was sequenced and identified as being in the HA clade 6b1.A.5a.2, which is the same genetic group as the vaccine virus, A/Victoria/2570/2019. All confirmed influenza B viruses (7/10) were of the B/Victoria/2/87-lineage and 4/4 sequenced viruses fell into the HA clade V1A.3a.2. This is genetically distinct from the B/Victoria vaccine virus B/Washington/02/2019, but three isolates tested in HI were antigenically similar.

Risk of importation of influenza

Chains of transmission within family traveling groups were observed during quarantine resulting in detections as late as day 9 and three cases continued to test positive as late as days 11 and 12 (Supplementary Figure 1), albeit with high Ct (cycle threshold) values indicating low viral load. We used a Bayesian framework to assess the risk that these travellers might leave quarantine still infectious. Only travellers arriving on direct flights from New Delhi to Darwin for the period 3

February 2021 to 22 September 2021 were considered. Under the assumed model, when quarantine was 14 days, there was 0% probability that an infectious traveller would exit quarantine still infectious and potentially initiate onward transmission (Figure 4), as observed in Darwin. When the quarantine period was reduced to 7 days, with influenza testing on day 5, this probability increased to 49% (95%CI:47,52), and without testing increased to 91% (95%CI:90,92). Without quarantine, there is a 100% probability of a traveller being infectious in the community.

Discussion

Our observations of influenza detections in quarantine are relevant beyond Australia for several reasons. First, the number of passengers arriving in a port like Darwin is very small. Therefore, the implication for countries that have a much higher volume of passengers is that influenza had probably been introduced undetected on a number of occasions. Although our study focussed on passengers from India, at the end of the study period influenza case numbers were also increasing in the UK (17) and the US (18), where quarantine requirements were less strict. Thus, it seems likely that importations had been occurring in those countries for some time before detection by surveillance systems.

Second, the detections of influenza among travellers arriving from India identified potential high circulation of influenza at a time when national reporting suggested circulation was limited. During the early part of 2021, India was managing a large outbreak of SARS-CoV-2 Delta infections, which would have limited the country's capacity to conduct surveillance for other diseases. Detections among quarantine travellers could therefore have provided additional data

on influenza circulation in that country that may now have been known to local authorities and which could be used by other countries in their surveillance of returned travellers.

Third, we were able to use the information about cases and total passengers to estimate the likelihood of an influenza case exiting quarantine still infectious. Although the model we used only explored a limited number of assumptions, this type of information could inform expectations about the re-circulation of influenza, and could be applied to other infectious pathogens. It is important to note that not every infectious influenza case will initiate an outbreak (19). Ongoing pandemic mitigation strategies like mask wearing and social distancing may help limit the spread of influenza more effectively than SARS-CoV-2 given its lower effective reproduction number (20, 21). However, quarantine policies that focus exclusively on the importation risk of SARS-CoV-2, like those in Australia (22) and most other countries, did not consider preventable importations of other infectious respiratory pathogens, like influenza, the burden of which can be substantial (23, 24). Given continued circulation of SARS-CoV-2 at the time borders were reopened, the risk of dual epidemics of influenza and SARS-CoV-2 was inevitable. Models that attempted to forecast the impact of relaxing border restrictions both in Australia (25, 26) and elsewhere could have incorporated renewed influenza circulation to create a more completed picture of healthcare system overwhelm as co-circulation of these two viruses carries a substantial burden.

Finally, the identification of influenza viruses globally was extremely limited in 2020 and 2021 (6) which made selection of representative antigens for influenza vaccines challenging (5). By testing all passengers in quarantine, we were able to obtain representative viral isolates that could be used for influenza vaccine development, and two viruses from passengers arriving in Darwin were listed as WHO-recommended vaccine viruses for the A(H3N2) component of the 2022

influenza vaccine (5). Thus, in future pandemics, the testing of travellers in quarantine can provide an important source of viral samples for influenza vaccine development when pandemic mitigation strategies have suppressed transmission.

Our study was limited to passengers arriving on government-supported repatriation flights in Darwin. We were unable to include cases from other Australian ports, which received a large number of private flights, because they did not test for influenza. Their inclusion may have permitted exploration of the risk of importation of influenza from other parts of the world, as Darwin only received repatriation flights from a limited number of countries. Nevertheless, our model demonstrates that importation was a risk, and prior application of the model to SARS-CoV-2 (12) has demonstrated the variation that might also be expected for influenza.

In conclusion, influenza testing of repatriated travellers in Darwin enabled identification of candidate vaccine viruses and alerted us to influenza activity in a common port of origin. During a pandemic, failing to test quarantined travellers for influenza, represents a missed opportunity for enhanced surveillance to better inform public health preparedness.

NOTES

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FIGURE LEGENDS

Figure 1. Influenza activity among returned travellers arriving in Darwin on repatriation flights from India, 2 January – 14 October 2021. (A) The number of cases detected per week in Darwin; (B) the percent of passengers positive for influenza per flight; (C) the number of notifications of influenza notified by the Indian National Influenza Centre to FluNet, the World Health Organization's web-based tool for influenza virological surveillance. Note that only detections in Darwin to 11 October 2021 are included and further detections may have occurred after this date. The relatively low number of influenza detections in the first half of 2021 in India may be the result of resources being redirected to SARS-CoV-2 testing or could be associated with the location of the National Influenza Centre, which is located in Pune not New Delhi.

Sources: https://covid19.who.int/data, https://www.who.int/tools/flunet

Figure 2. Network plot showing the potential transmission of viruses on flights. Edges (lines) linking nodes (cases and non-cases) identify travelling groups and show the presence of infections among lone travellers as well as travelling groups on the same flight. Several clusters show the arrival of passengers infected with different types/subtypes of influenza on the same flight (e.g. IND79, IND88 and IND101), suggesting co-circulation of A(H1N1)pdm09, A(H3N2) and B/Victoria in India during the study period. Detections of influenza sometimes occurred in single travellers (e.g. IND98), suggesting potential inflight or in-transit transmission.

Figure 3. Phylogenetic tree showing clustering of A(H3N2) viruses identified from travellers by flight and travelling group. Virus names are coloured by travelling group and tips are coloured

by flight. Similarities in the haemagglutinin gene among viruses from unrelated passengers on the same flight (e.g. IND69) suggest possible in-flight transmission. However, there were also highly similar viruses recovered from passengers travelling on different flights many months apart (e.g. IND30 & IND69). Note that two viruses are included for A/Darwin/6/2021 and A/Darwin/29/2021, which were viruses collected on different days but which showed no genetic variation over time at the amino acid level.

Figure 4. Importation risk of infectious travellers: The number and probability of released infected travellers based on 2000 simulations. Dot represents the median the vertical line represents the inter-quartile range.

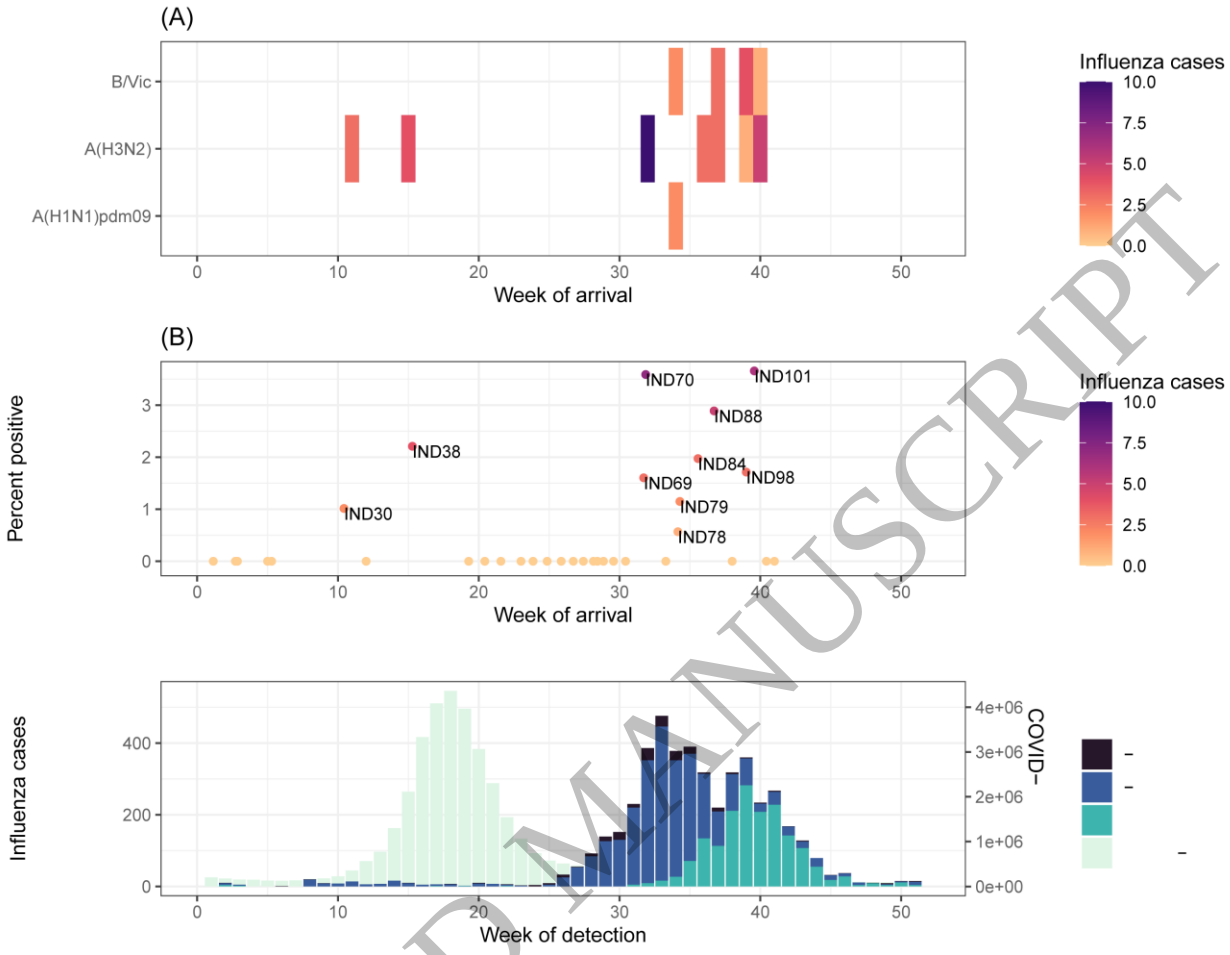

Figure 1
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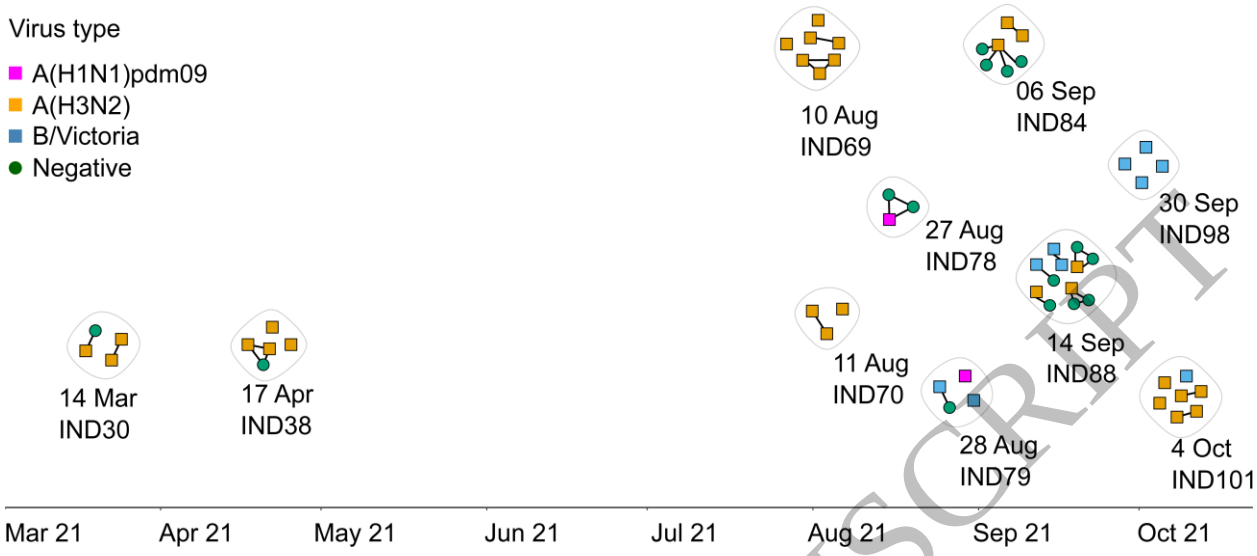

Figure 2
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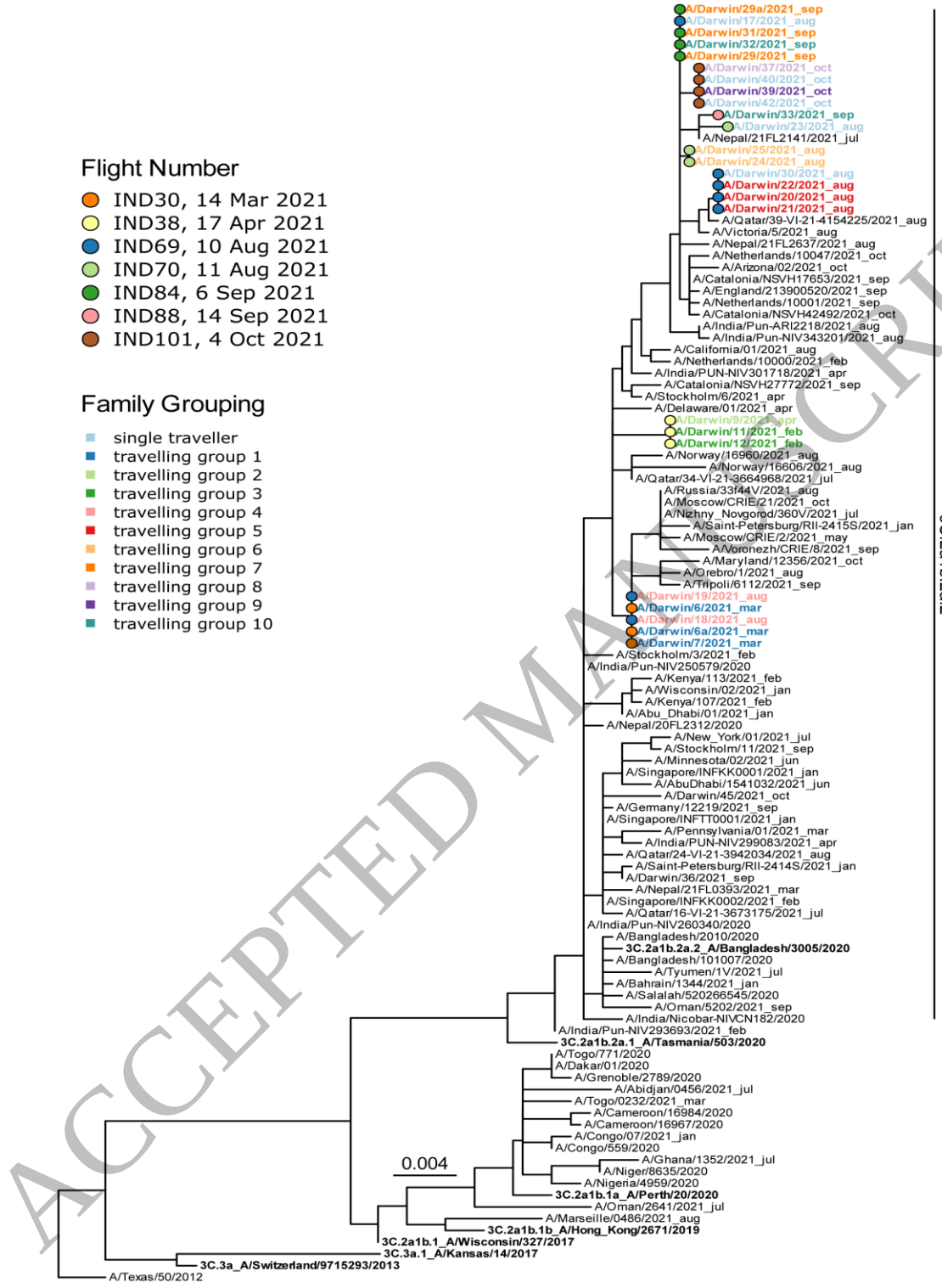

Figure 3
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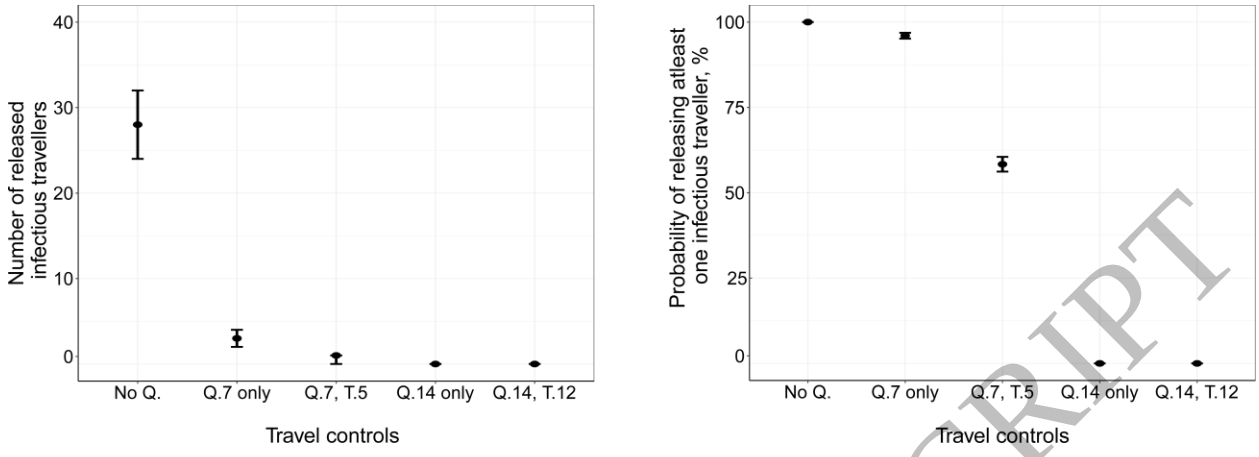

Figure 4
165x58 mm (x DPI)