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Suitability of aircraft wastewater for pathogen detection and public health surveillance

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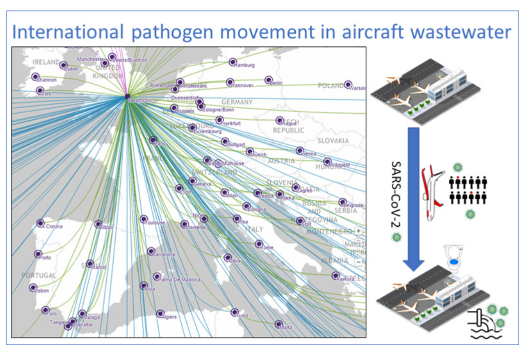
HIGHLIGHTS

- Air travel is important for long distance human pathogen transport.
- We surveyed the toilet habits of individuals on short- and long-haul flights.
- We estimate that wastewater captures 8–14 % of infected individuals entering the UK.
- Wastewater may prove useful for public heath surveillance at national borders.

GRAPHICAL ABSTRACT



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ABSTRACT

International air travel is now widely recognised as one of the primary mechanisms responsible for the transnational movement and global spread of SARS-CoV-2. Monitoring the viral load and novel lineages within human-derived wastewater collected from aircraft and at air transport hubs has been proposed as an effective way to monitor the importation frequency of viral pathogens. The success of this approach, however, is highly dependent on the bathroom and defecation habits of air passengers during their journey. In this study of UK adults ($n = 2103$), we quantified the likelihood of defecation prior to departure, on the aircraft and upon arrival on both short- and long-haul flights. The results were then used to assess the likelihood of capturing the signal from infected individuals at UK travel hubs. To obtain a representative cross-section of the population, the survey was stratified by geographical region, gender, age, parenting status, and social class. We found that an individual's likelihood to defecate on short-haul flights (< 6 h in duration) was low (< 13 % of the total), but was higher on long-haul flights (< 36 %; > 6 h in duration). This behaviour pattern was higher among males and younger age groups. The maximum likelihood of defecation was prior to departure (< 39 %). Based on known SARS-CoV-2 faecal shedding rates (30–60 %) and an equal probability of infected individuals being on short- (71 % of inbound flights) and long-haul flights (29 %), we estimate that aircraft wastewater is likely to capture ca. 8–14 % of SARS-CoV-2 cases entering the UK. Monte Carlo simulations predicted that SARS-CoV-2 would be present in wastewater on 14 % of short-haul flights and 62 % of long-haul flights under

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current pandemic conditions. We conclude that aircraft wastewater alone is insufficient to effectively monitor all the transboundary entries of faecal-borne pathogens but can form part of a wider strategy for public heath surveillance at national borders.

1. Introduction

The role of international air travel in the initial spread of SARS-CoV-2 and the subsequent introduction of new variants has been well documented during the COVID-19 pandemic (Khanh et al., 2020; Lodder and de Roda Husman, 2020; Murphy et al., 2020; Hu et al., 2020; Swadi et al., 2021; Toyokawa et al., 2022). This has led to the implementation of a range of non-pharmaceutical interventions to limit the spread of the virus in the airport terminal (e.g. social distancing, point-of-departure testing), within the aircraft (e.g. face coverings) and upon arrival (e.g. mass quarantining facilities; Bielecki et al., 2021; Ohlsen et al., 2021; Yokota et al., 2021). Despite these measures, however, many individuals infected with SARS-CoV-2, who are both aware or unaware of their infection status, are still undertaking transnational journeys (Myers et al., 2020; Qahtani et al., 2021). For example, in the UK, estimates suggest that between 1 and 5 % of individuals entering governmental quarantining facilities on arrival from red list countries test positive for COVID-19 within the isolation period (UKHSA, 2022a). Although viral transmission could occur on the aircraft, the frequency of this is believed to be relatively low, despite well-documented cases where it has occurred (Pang et al., 2021; Zhang et al., 2021). Rather, the high rate of import suggests that point of departure testing is prone to significant error (i.e. false-negatives), that an individual's infection status is being erroneously reported, or that passengers are in the early stages of infection where low viral titers are too low and thus evade detection (Ahmed et al., 2022a). Identification of these variants entering via aircrafts provides potential insight on viral seeding into a nation.

Mirroring the evolutionary course of other viral pathogens, there will be a transition from pandemic to endemic COVID-19. To ensure that the prevalence of the disease is kept low, the continued surveillance of SARS-CoV-2 evolution and global distribution will be required for the foreseeable future, by monitoring the emergence and spread of novel variants (Daon et al., 2020; Sharun et al., 2020). A coordinated international effort is needed to ensure that we are better placed to identify early, and limit the spread of disease should another pandemic occur (Dollard et al., 2020; Jernigan et al., 2020; Pham et al., 2021). It has also been suggested that global surveillance should be extended to include other respiratory and enteric viral pathogens of public health concern (Kuan and Chang, 2012; Yang et al., 2010).

Although SARS-CoV-2 is a respiratory pathogen, it is well documented that it also multiplies within the gastrointestinal tract (Kipkorir et al., 2020; Andrews et al., 2021). This leads to the virus being shed in feces and very occasionally urine (Jones et al., 2020; Bwire et al., 2021; Crank et al., 2022). Although the virus is shed in feces, this typically occurs at much lower rates than in respiratory droplets and is not thought to be infectious (Jones et al., 2020; Dancer et al., 2021). Urinary shedding is normally associated with multi-organ infections, particularly in very severe cases (Perrella et al., 2021) and we hypothesise that individuals with this type of very clear infection would not be travelling on an aircraft, rather they would be in a healthcare facility being treated. Consequently, urine was not considered as a major source of SARS-CoV-2 RNA in aircraft wastewater in this study.

The loss of bodily fluids (e.g. saliva, vomit, feces, urine) has provided an opportunity to monitor SARS-CoV-2 RNA levels in human-derived wastewater as a measure of community-level COVID-19 prevalence (Hillary et al., 2021; Wade et al., 2022). In areas where most people are connected to a centralised wastewater treatment plant it can provide a relatively unbiased estimate of disease prevalence capturing asymptomatic, pre-symptomatic and symptomatic infections (Tran et al., 2021). In an international air travel context, this wastewater-based surveillance approach may offer the potential to determine relative rates of COVID-19 passage through travel hubs (Hjelmsø et al., 2019; Lodder and de Roda Husman, 2020). The approach is also particularly suited to protozoal and viral diseases where diarrhoea is a primary symptom (e.g., giardia, norovirus, rotavirus, enterovirus; Lopman, 2011; Nordahl Petersen et al., 2015; Hjelmsø et al., 2019; Jian et al., 2021). This surveillance approach is also supported by previous studies showing that aircraft-derived wastewater contains a wide range of antimicrobial resistance genes as well as SARS-CoV-2 RNA (Heß et al., 2019; Albastaki et al., 2021; Amoruso and Baldovin, 2021; Ahmed et al., 2022a, 2022b). This has led to the possibility of using wastewater-based epidemiology (WBE) at international borders for public health surveillance. The success of wastewater as an indicator of transboundary pathogen transfer, however, clearly relies on an individual's relative use of toilet facilities prior to departure, during a flight, or upon arrival. Knowledge of this is therefore vital to understanding the proportion of passengers that can be captured using wastewater monitoring. This information would form a key part in the design of optimal sampling strategies, which is particularly important considering the annual 42 million flights occurring globally and the need to sample these in a strategic and cost-effective way. While several studies have investigated passenger perception of toilet cleanliness on airplanes in a health risk context (Suki, 2014; Munoz et al., 2019; Park and Almanza, 2020) and pathogen spread from aircraft toilets (Li et al., 2022), no comprehensive studies have been undertaken on toileting behaviour in an aircraft and terminal setting.

Data on passenger bathroom usage is critical to develop an understanding of the utility for WBE to monitor importation, or seeding, of SARS-CoV-2, its variants, and other pathogens (Dancer et al., 2021). A key question to gather this knowledge is whether people use the bathroom to urinate and/or defecate prior to departure, during the flight, or upon arrival? Understanding this, and the potential for people to fly when noticeably symptomatic, will inform the use cases and the sampling strategies for these. With a focus on UK air travelers, in particular those that have flown during the pandemic, the primary aim of this study was to evaluate passenger behaviour in relation to toilet habits within an international air travel setting. A secondary objective was to identify whether this behaviour was linked to demographic factors, such as age and gender. Lastly, the information was used to critically evaluate the suitability of wastewater testing of aircraft or at air travel hubs as a mechanism for future disease surveillance.

2. Methods

2.1. Survey design

We commissioned the ESOMAR accredited market research company YouGov (YouGov Ltd., London, UK; Twyman, 2008) to carry out a cross-sectional survey involving 2103 participants recruited from their research panel ($n = 800{,}000+$ UK adults). Individuals were deemed eligible if they were aged 18 years or older and living in the UK. Comparisons of opt-in internet panels with traditional stratified random sample interview and random digit dial techniques conclude that the biases introduced by this type of methodology are small, and in general are more than offset by the much larger sample sizes the internet-based methodology permits (Hill et al., 2006). The random error on a sample of 2000 individuals is estimated to be up to 2 %. Quota sampling was used, based on age, gender and UK region, to ensure that the sample was broadly representative of the UK general population (Table S1). All participants provided socio-demographic information. If participants had never taken a short-haul flight ($n = 231$) or long-haul flight ($n = 483$) they were excluded from the subsequent analysis. Participants were invited to participate in the survey by an email with the subsequent survey conducted on-line via the YouGov data portal. Active sampling restrictions were put in place to ensure that only people contacted and registered with YouGov were allowed to participate. Interviewees were renumerated with points which were redeemable.

The survey consisted of 15 closed-ended questions, with 7 of the questions addressing personal behaviour when undertaking air travel and 8 questions collecting demographic information (age, gender, social grade, employment status, highest educational or professional qualification, parenting and marital status, social media use). Definitions of the social grades are shown in Table S2. The questionnaire was designed by the research team, consisting of environmental microbiologists, public health specialists and social scientists, based on the study objectives and incorporating information from previous studies on similar topics. The draft questionnaire was then tested on an expert panel, a panel of non-experts, a local ethics committee and finally refined by YouGov prior to deployment. Questions about likely behaviour were measured using a 5-point Likert scale (e.g. never to always). We also asked if they had travelled abroad by plane since the start of the COVID-19 pandemic and whether they had ever tested positive for COVID-19. Participants were asked for their postcode to determine indices of multiple deprivation (IMD) and their social grade. Comparisons between groups was made using chi-squared tests using $P < 0.05$ as the cut-off for statistical significance. Monte Carlo simulations ($n = 50{,}000$) were used to predict the likelihood of capturing infected individuals via toilet use on short- and long-haul planes. All simulations were performed in Minitab Workspace v1.3 (Minitab Inc., State College, PA, USA). Faecal shedding rate was estimated using a normal distribution using parameter estimates from 11 independent published studies (shedding rate mean ± SEM, 45.9 ± 16.8 %). The probability of toilet use was described using a triangular distribution using the survey data for short- and long-haul flights. In addition, Monte Carlo analysis was used to predict the likelihood of an infected passenger being on a plane and the subsequent detection frequency of SARS-CoV-2 in aircraft wastewater. This used the same shedding and toilet use data as above, but additionally incorporated estimates of aircraft seating capacity, flight occupancy rate, and national prevalence of COVID-19 (Table S3).

Ethical approval for this study was granted by the Bangor University College of Environmental Sciences and Engineering Ethics Committee (Approval Number: COESE2020EG01A). The survey questions and responses are available as an open-access data archive on Zenodo (Jones et al., 2022).

3. Results

Overall, our data revealed that a maximum of 13 % of individuals are likely to defecate on a short-haul flight (< 6 h in duration; Fig. 1A). Further, this response shows a small but significant gender effect ($P = 0.005$) and positive relationship with age ($P = 0.012$) although there was no separation based on social grade ($P > 0.05$) or family status ($P > 0.05$). In contrast to short-haul flights, the likelihood of defecating on a long-haul flight (> 6 h in duration) was much greater (ca. 36 % of the total) with more respondents suggesting that this might occur sometimes (Fig. 1B). Further, this showed a strong gender bias with males more likely to defecate than females ($P < 0.001$) and a negative relationship with age class ($P < 0.001$). However, no clear relationship was apparent with social grade or family status ($P > 0.05$).

The likelihood of defecation within the airport terminal prior to boarding a flight (39 % of the total) was much greater than those associated with the flights themselves (Fig. 2A). Again, this showed a major gender bias with more males (45 %) likely to defecate in comparison to females (33 %; $P < 0.001$) and greater likelihood in younger age groups ($P < 0.001$). There was, however, no relationship of defecation prior to departure and either social grade or family status. In terms of likely defecation behaviour upon arrival at the destination, but still within the airport terminal, 71 % of respondents said that this was unlikely to occur with ≤5 % responding that this would occur often (Fig. 2B). The likely frequency was slightly higher in males ($P < 0.001$) and showed a negative relationship with age ($P < 0.001$) although there was no link to social grade and family status.

When asked about their previous experience of returning back to the UK on an international flight, 23 % of respondents indicated that they had previously boarded a flight while feeling ill (e.g. feeling sick, diarrhoea, headache etc.; Fig. S1). Although not affected by social grade or gender, a greater proportion of the younger age groups (ages 18–44, $n = 833$) had travelled while ill in comparison to those in the older age groups (ages 44+, $n = 1076$).

4. Discussion

4.1. Theoretical considerations in using aircraft wastewater for public health surveillance

Although airport wastewater has been proposed as a mechanism for tracking pathogen entry at national borders (Heß et al., 2019; Amoruso and Baldovin, 2021), its success is critically dependent upon a wide range of factors which ultimately makes its implementation complex. These may include physical infrastructure issues (e.g., access to sewer lines, sewer connectivity, security), economic factors (e.g., cost of sampling and analysis), operational factors (e.g., frequency of cleaning, addition of disinfectants), ethical issues, the types and times of flights entering the terminal (e.g., domestic vs. international, short- vs. long-haul), how often wastewater is offloaded from aircraft and the behaviour and infection status of passengers and airport staff (e.g., toilet habits). Here we focused on the most important factor, in our view, which is the potential to capture a representative sample of faecal material from a cohort of passengers. To our knowledge, this is the first estimate of toilet behaviour in a range of airport surveillance settings.

Although the focus is currently on the use of faecal material to monitor the frequency at which new variants of SARS-CoV-2 enter a country, wastewater may also contain a wealth of information on other respiratory and enteric pathogens (Ahmed et al., 2022b). Unfortunately, however, quantitative information on the rates of viral shedding in faecal material (and other bodily fluids) is not well known as it depends on the point in the infection cycle as well as the health status and age of individuals (Jones et al., 2020). Based on animal models (e.g. hamsters), shedding rates of SARS-CoV-2 may also change over time in response to the introduction of new variants (e.g. delta vs. omicron; Yuan et al., 2022), however, it should be noted that the frequency of COVID-19 symptoms such as diarrhoea and nausea have shown no major temporal trend over the last 2 years (UKHSA, 2022b). In the case of COVID-19, it is estimated that between 30 and 60 % of infected individuals shed SARS-CoV-2 in appreciable quantities in faecal material (Chen et al., 2020; Huang et al., 2020; Parasa et al., 2020; Zheng et al., 2020). This wide uncertainty makes it difficult to relate the levels of SARS-CoV-2 RNA in aircraft wastewater to the actual number of infected individuals. Wastewater analysis at borders therefore needs to rely on the identification of viral lineages/variants to identify case numbers, assuming that each infected group of individuals carries a distinct lineage (du Plessis et al., 2021; Tang et al., 2021). In this study, we found that a maximum of 13 % of individuals said they were likely to defecate during a short-haul flight. Assuming a SARS-CoV-2 shedding rate in individuals of 30 to 60 %, this implies that wastewater samples would fail to capture potential COVID-19 infections from a large proportion of the passengers on this type of inbound flight, making it largely ineffectual for mass surveillance purposes. A Monte Carlo analysis indicated that mean probability (±SD) of capturing an infected person via wastewater surveillance on a short-haul flight was 8.0 ± 5.0 % (Fig. 3A). In the case of enteric viruses (e.g. norovirus, enterovirus) where the likelihood of shedding into a toilet may be closer to 100 %, then the chances of capturing infected individuals using wastewater are much greater (Lopman, 2011). Further, if we assume the best-case scenario that all infected individuals will use the toilet facilities (e.g. due to vomiting and diarrhoea; Graf et al., 2012), then the failure rate of wastewater to capture individuals would be much lower, especially as infected individuals are likely to

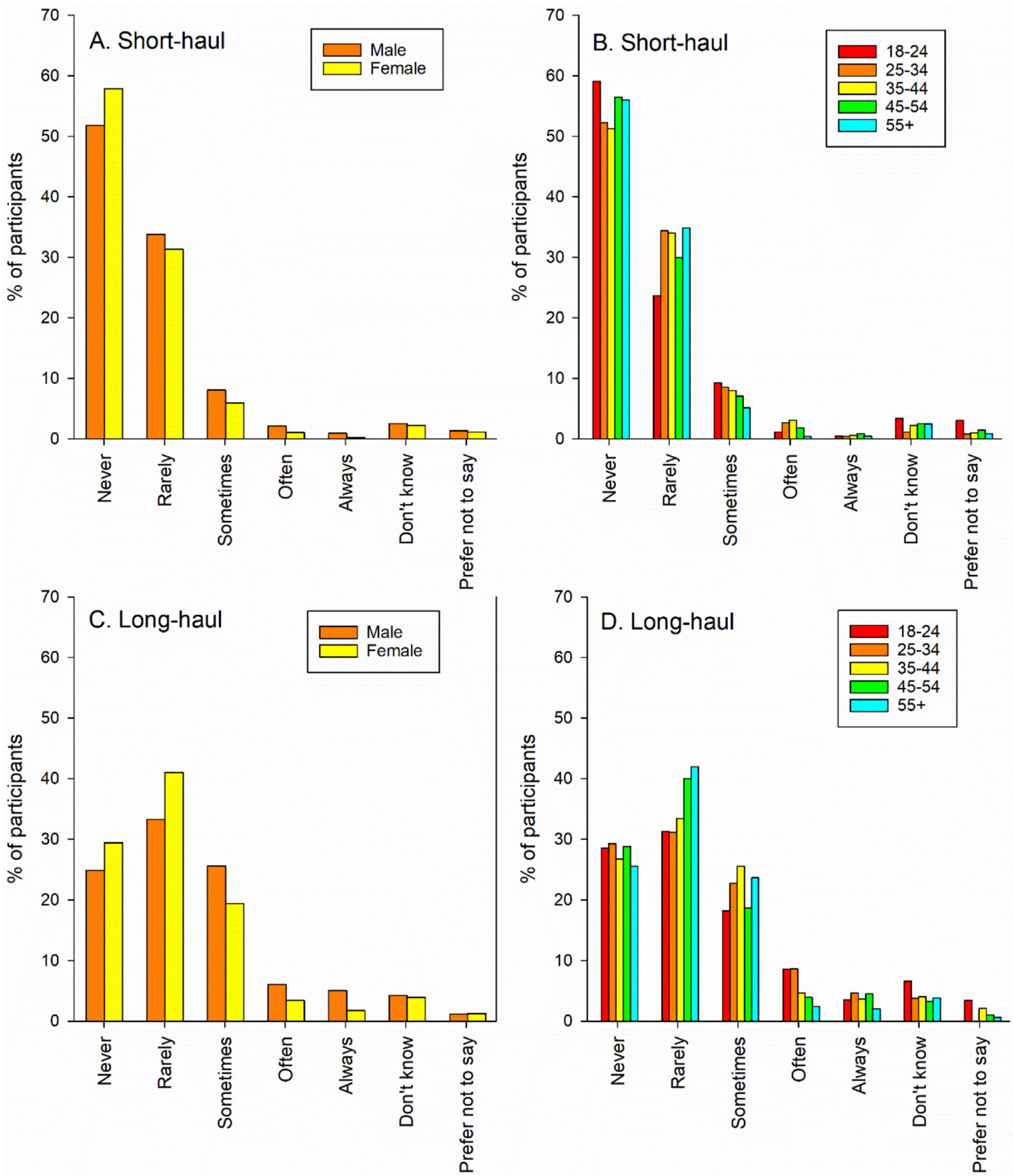

Fig. 1. Evaluation of the likelihood that individuals would use the aircraft toilet to defecate on either a short-haul flight (A, B upper panels) or long-haul flights (C, D lower panels), stratified by either (A, C) gender, or (B, D) age. Sample size (short haul, n = 1872; long-haul n = 1620).

board the plane; Fig. S1). A caveat to this would be individuals carrying the infection but who are taking anti-diarrhoea or -nausea medications (e.g. loperamide) and the asymptomatic carriage of enteric viral pathogens (e.g. 7–12 % of the population are asymptomatic for norovirus; Qi et al., 2018; Phillips et al., 2010).

The situation for long-haul flights was slightly better than for short-haul flights due to the increased likelihood of visiting the toilet. Using a Monte Carlo simulation, we estimated the mean probability (±SD) of capturing a person infected with SARS-CoV-2 using aircraft wastewater was 13.9 ± 8.5 % (Fig. 3B). This is likely to be highly correlated to the increased time spent on the plane as defecation frequency ranges from 0.74 to 1.94 stool motions per 24 h (Bloom et al., 1993; Rose et al., 2015). One caveat of our analysis is that one of the symptoms of SARS-CoV-2 infection is diarrhoea which might slightly increase the frequency of toilet use and capture of cases, however, this symptom is typically only observed in 7 ± 2 % of clinically reported COVID-19 cases (Parasa et al., 2020; Jones et al., 2020; Aiyegbusi et al., 2021; Cloete et al., 2022; UKHSA, 2022b; Table S3). Another caveat of our study is that we only surveyed the behaviour of UK citizens. This captures ca. 90 % of border entries into the UK (mainly from UK citizens returning from foreign holidays; Osborn, 2020), however, we acknowledge that cultural and gender differences in defecation behaviour are also likely (Sandler and Drossman, 1987; Hardacker et al., 2019; Reynolds et al., 2020). We also recognise that the demographic and citizenship status of passengers entering the UK will also vary during the year (ABS, 2022; UKHO, 2022).

4.2. Practical considerations for the use of aircraft wastewater for public health surveillance

There are several potential WBE use cases for air travel. When the incidence of SARS-CoV-2 in the population is very low, its use as a screening

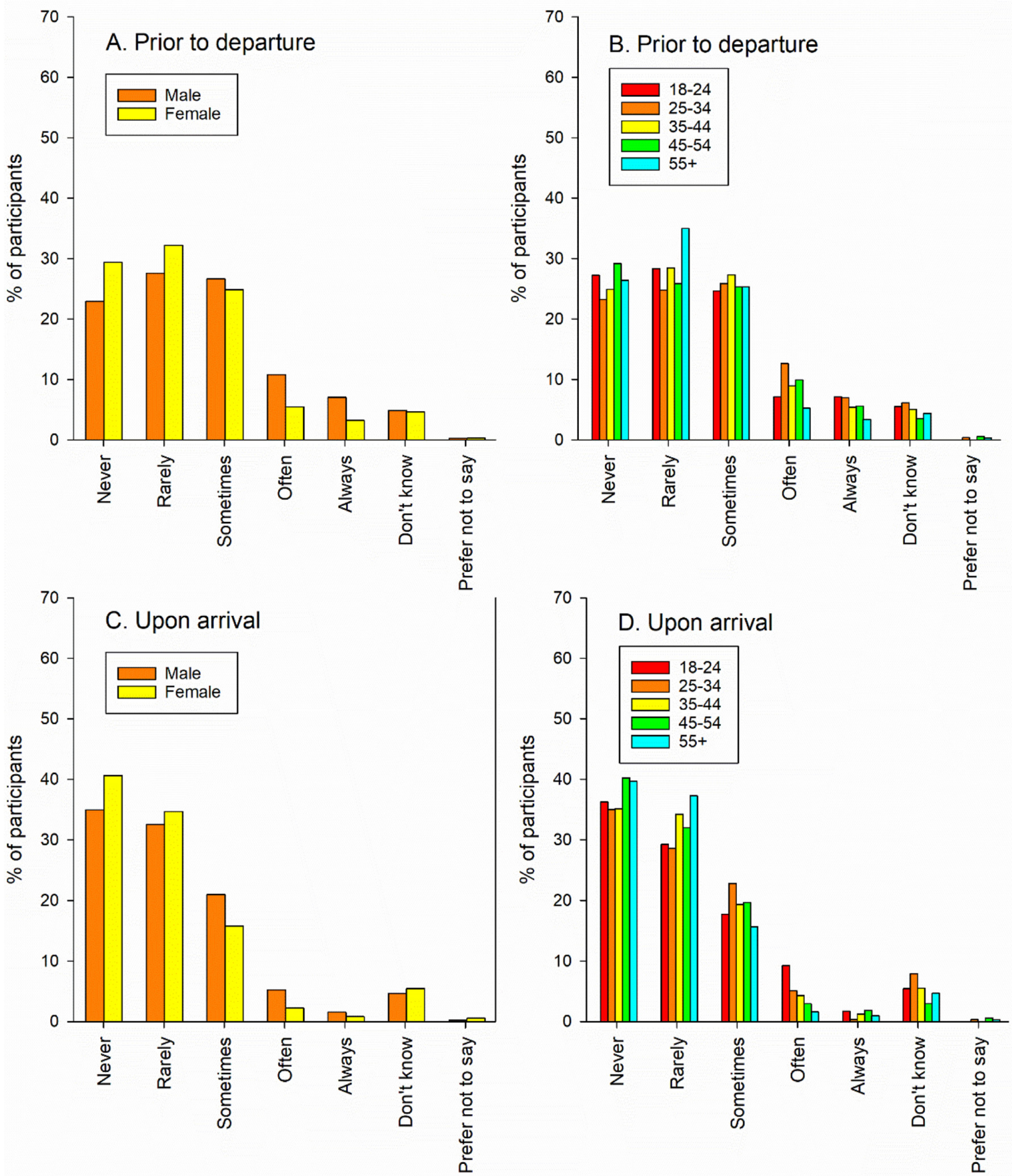

Fig. 2. Evaluation of the likelihood that individuals would use the airport terminal toilets to defecate either before boarding the plane (A, B upper panels) or upon landing in the UK (C, D lower panels), stratified by either (A, C) gender, or (B, D) age (n = 1909).

tool for a single flight, even if followed up with individual testing when positive, may be somewhat limited. This is due to the lower likelihood of individuals using toilets in flight, and uncertainties in the distribution of faecal shedding. Conversely, if the incidence of SARS-CoV-2 is high, then wastewater has a better chance to capture some of the infected individuals (Ahmed et al., 2022a, 2022b). A caveat would be that faecal shedding often persists for much longer than shedding in respiratory droplets (deemed the infectious period), which would lead to the potential detection of false positives (Zhang et al., 2021). When sampled as a time series, wastewater prevalence data has a strong potential to provide insight on the rate or variant or pathogen seeding into a country by aggregating across all flights, or potentially aggregating across routes of interest. With the higher likelihood of pre-departure bathroom use, there is potential value in cross-nation collaboration of sampling strategies to maximise the understanding of international transmission of pathogens.

Here we place our results in a UK context, however, the principles are directly applicable to many other nations worldwide. Of the 1.3 million flights arriving at major UK airports each year (pre-pandemic), 6 % are classed as domestic, 67 % are international short-haul and 27 % international long-haul (Fig. S2, Table S4). Assuming the primary focus will be on the ca. 350,000 long-haul flights each year, it will be impractical to sample wastewater from each flight. It is likely that collection of aggregated wastewater samples will therefore be required to capture the temporal resolution needed for effective surveillance. This strategy is ideally suited to airports with terminals dedicated to long-haul carriers (e.g. Heathrow Terminal 3), however, this separation between international and domestic

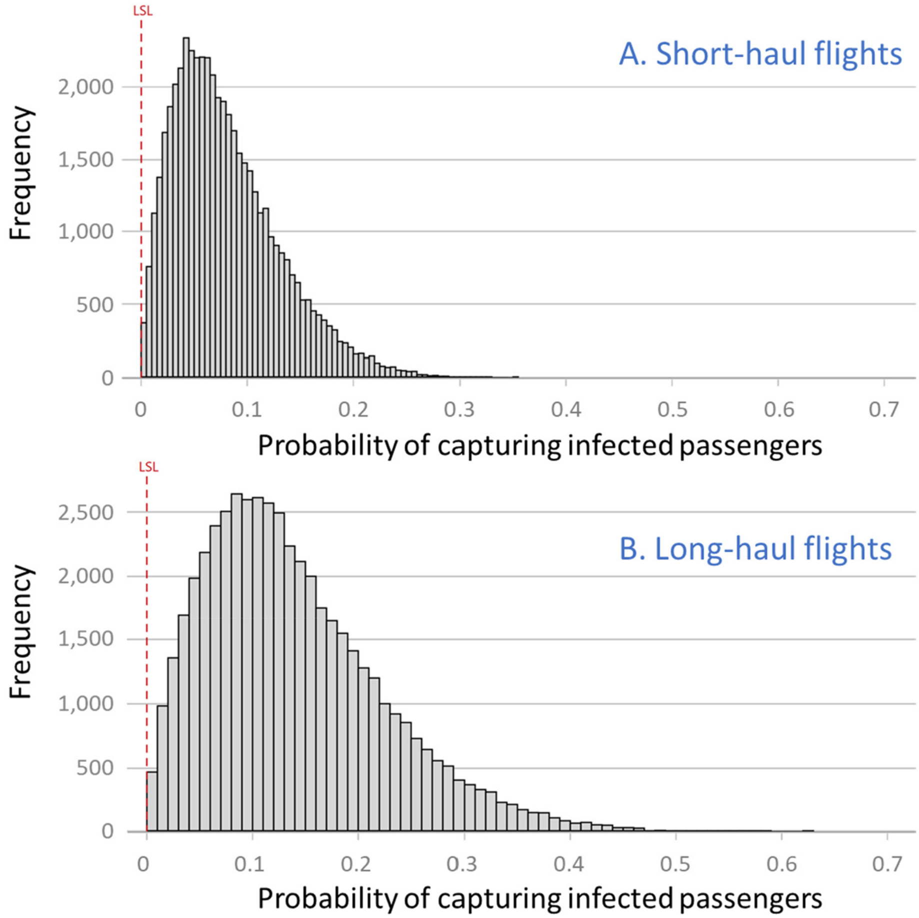

Fig. 3. Frequency distribution for the Monte Carlo simulation (n = 50,000) describing the probability of capturing individuals infected with SARS-CoV-2 through toilet use on either (A) short-haul, or (B) long-haul flights. LSL designates the lower boundary limit.

passengers does not occur at most UK airports. It should also be noted that short-haul flights often visit a number of locations around European travel hubs prior to discharging their waste in the UK. Thus, wastewater samples may contain false positives (i.e. faecal material collected between 2 European cites, rather than on the leg entering the UK). The rate of carry-over (i.e. cross-contamination) between successive waste unloading events also remains unknown. From this perspective, a better understanding of the rate of degradation of SARS-CoV-2 RNA in aircraft wastewater tanks and the efficiency of tank emptying and cleaning/disinfection would be useful.

Our analysis clearly indicates that more people are likely to defecate prior to boarding the aircraft. It is likely that many will also defecate prior to leaving for the airport (Reynolds et al., 2019). Although, it is much easier to sample wastewater from airport terminals than from individual flights, this is not without its problems. For example, sewage from departure and arrival halls is often not well segregated within the sewer network. It is also likely contains bodily fluids from terminal workers (e.g. cleaning, retain and administrative staff) and domestic passengers, not just international passengers. It is likely, therefore, that many pathogens detected could be exports rather than imports or from transit passengers who do not pass though passport control and enter the country. This further highlights the potential benefit of international collaborations on sampling strategies and analysis. Although imports and exports can theoretically be separated by next generation sequencing, this can be difficult to achieve when trying to identify rare lineages. Further, the presence of chemical additives in aircraft water (e.g. disinfectants and deodorisers), its higher ionic strength and total suspended solid load (Table S5) may also represent difficulties for the recovery of genetic material and subsequent downstream RT-qPCR and genomic sequencing. The development of internationally accepted and validated methods for DNA and RNA recovery from aircraft wastewater is therefore warranted.

One area for further research is the study of wastewater at major international air travel hubs. This would be of particular interest where there are large quantities of transit passengers. For example, Dubai International Airport has over 90 million passengers annually, predominantly transit/connecting passengers (63 % of the total), and is connected to 93 countries. In this type of situation, the sequencing of SARS-CoV-2 in wastewater would be highly beneficial to estimate the number of unique lineages present. This would provide an estimate of the global migration frequency of SARS-CoV-2 and other diseases. This type of surveillance approach will also be facilitated by recent advances in viral sequencing from wastewater samples and downstream bioinformatics (Karthikeyan et al., 2022).

One of the limitations of this study is that we did not capture the influence of flight times on defecation habits and believe these should also be considered, as most people defecate (~60 %) in the morning (Heaton et al., 1992). In addition, other routes of entry of SARS-CoV-2 RNA into aircraft wastewater (e.g. saliva, vomit) and associated human behaviors (e.g. spitting in the sink, cleaning teeth, nasal tissue disposal) were not investigated here.

4.3. Validation of the survey results findings

Our self-reporting survey approach followed those used previously on toileting habits and hygiene perception (Palmer et al., 2018; Wu et al., 2019; Park and Almanza, 2020; Reynolds et al., 2020). In terms of validating the findings presented here, another approach would be to undertake 'intercept' surveys of toilet behaviour upon passenger arrival. This is likely

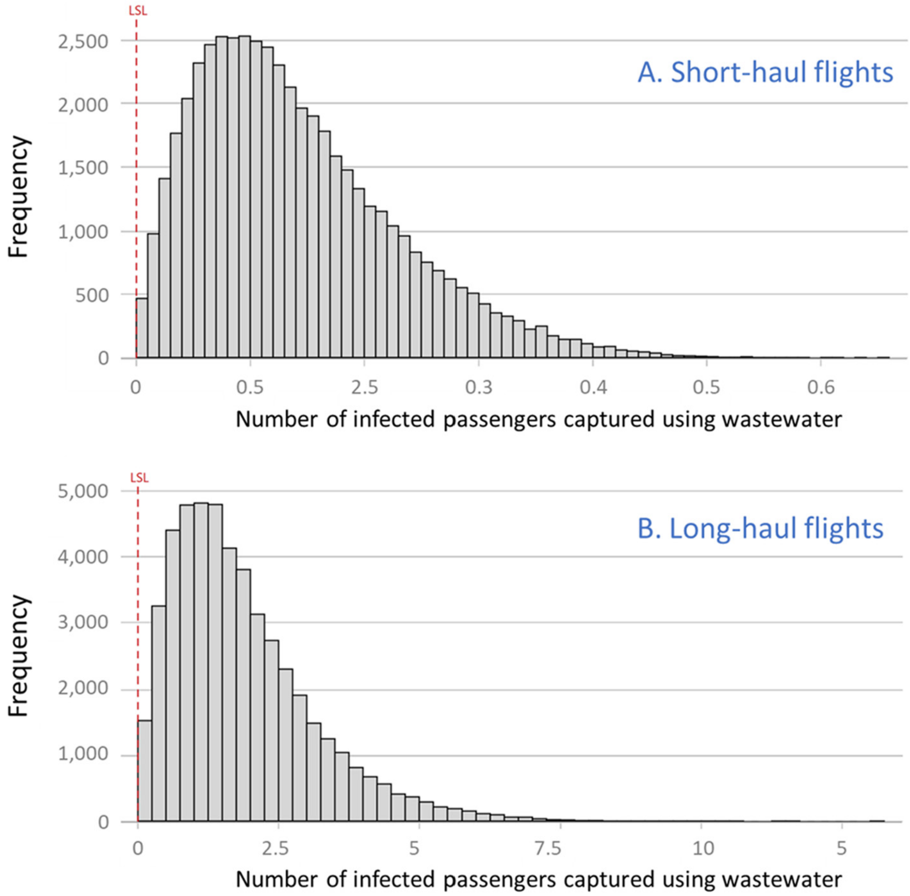

Fig. 4. Frequency distribution for the Monte Carlo simulation (n = 50,000) describing the number of infected individuals captured by wastewater on either international (A) short-haul or (B) long-haul flights. LSL denotes the lower boundary limit. Note the different x-axis and y-axis scales.

to reduce avidity and recall bias (Lewin et al., 2021). It should be noted, however, that the intercept survey approach can lead to significant bias in reporting, particularly when asking questions about bodily function (Nowell and Stanley, 1991; Bruwer et al., 1996; Ongena and Haan, 2022). Further, obtaining ethical approval, alongside logistical considerations (e.g. security clearance, time of sampling), and sampling bias (e.g. language, capturing variation in geographical and demographics) can also make on-site surveys difficult to implement.

An alternative way to validate our on-line survey, is to use Monte Carlo simulation to estimate (i) the number of infected individuals likely to be present on a flight, and (ii) the potential to capture these in wastewater based on our survey results. Using the assumptions for the number of infected individuals boarding flights, flight passenger numbers and seat occupancy (Table S3), we estimated the number of infected individuals that would be captured by wastewater surveillance on either a short-or long-haul flight. For a short-haul flight entering the UK we estimate that it would contain (mean ± SD) 1.8 ± 0.18 passengers carrying SARS-CoV-2 in the pre-symptomatic phase (Fig. S3A). However, we estimate that wastewater surveillance would only capture (mean ± SD) 0.14 ± 0.09 individuals infected with SARS-CoV-2 (i.e. suggesting that most flights would test negative, despite infected passengers being on board; Fig. 4A).

For long-haul flights, which contain more passengers than short-haul flights (Table S3), Monte Carlo simulations estimate that on average they would carry (mean ± SD) 5.4 ± 1.6 infected individuals (Fig. S3B). In this situation, wastewater-based surveillance would capture 0.75 ± 0.53 infected individuals (Fig. 4B). This predicted value for the proportion of flights whose wastewater would test positive for SARS-COV-2 (i.e. 75 ± 5 % of total flights) is very similar to the value of 69 % reported by Ahmed et al. (2022a) on repatriation flights arriving in Australia.

4.4. Conclusions

In summary, our findings demonstrate that the use of aircraft wastewater to evaluate the frequency of individuals infected with SARS-CoV-2 entering a country by air is likely to only capture a small proportion of the total inbound passengers infected with SARS-CoV-2. Despite this, the approach has particular merit for the surveillance of long-haul flights. However, it should be noted that most of the infections in the first COVID-19 wave originated from Europe (short-haul flights), whereas later variants arrived from long-haul journeys (du Plessis et al., 2021). If wastewater is used for transboundary pathogen surveillance it clearly needs to be undertaken in parallel with other complimentary approaches; preferably using technologies that capture a greater number of passengers in a non-invasive way (e.g. air filter cabin sampling; Korves et al., 2011). Looking beyond COVID-19, the use of wastewater has great potential as a medium in which to detect and quantify gastrointestinal pathogens, where diarrhoea is a primary symptom and where toilet use is likely to be higher. It also has merit for looking at the international flow of antimicrobial resistance genes. Wastewater-based epidemiology is likely to develop rapidly in the post-pandemic environment and the evidence presented here highlights a key knowledge area for research to ensure long-term utility and robustness for ensuring global public health resilience.

CRediT authorship contribution statement

DLJ, JMSG and KF designed the study and collected the data. JMR and DLJ analysed the data. DLJ wrote the first draft of the manuscript. All authors revised and approved the manuscript prior to submission.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Davey Jones reports financial support was provided by Natural Environment Research Council. Davey Jones reports financial support was provided by Engineering and Physical Sciences Research Council.

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Appendix A. Supplementary data

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