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Respiratory virus transmission using a novel viral challenge model: An observational cohort study

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SUMMARY

Objectives: Knowledge of Acute Respiratory virus Infection (ARI) is limited in relation to their substantial global burden. We completed a feasibility study of a novel method to study the natural transmission of respiratory viruses from young children to adults in hospital.

Methods: Between September 2012 and May 2015, we recruited healthy adults (contacts) and paediatric inpatients with ARIs (index) presenting to the University Hospitals Leicester NHS Trust, Leicester, UK. We took nose and throat swabs from all participants prior to controlled, 30 minute interactions between the children with ARIs and adult contacts. Contacts recorded symptoms and provided four nose and throat swabs over ten days post-interaction, which were tested for a panel of respiratory viruses to assess transmission.

Results: 111 interactions occurred between children with ARIs and adult contacts. Respiratory viruses were detected in 103 of 111 children (93%), most commonly rhinoviruses (RVs) (67 of 103, 65%). Transmission to an adult contact occurred in 15 (14·6%) of 103 interactions and was inversely associated with the contact being male (adjusted OR 0·12; 95% CI 0·02–0·72).

Conclusion: Using a novel methodology, we found that natural transmission of ARIs occurred in 15% of an infected child's contacts following a 30 minute interaction, primarily by RVs and when the contact was female. Our model has key advantages in comparison with human challenge studies making it well-suited for further studies of respiratory virus transmission, disease pathogenesis, and clinical and public health interventions to interrupt transmission.

Background

The recent emergence of Coronavirus Disease- 2019 (COVID-19), coupled with its morbidity and mortality, has renewed interest in how respiratory viruses are transmitted. Knowledge of the determinants of transmission is critical in informing non-pharmaceutical interventions and preventing spread. Whilst current concerns about infections due to SARS-CoV-2 predominate, other common causes of Acute Respiratory virus Infection (ARI) include rhinoviruses (RVs), enteroviruses (EV), respiratory syncytial viruses (RSV-A and RSV-B), influenza A and B (Flu), coronaviruses (CoV), adenoviruses (AdV), parainfluenza viruses (PIV), human metapneumovirus (HMPV), and bocavirus.[1,2] ARIs have huge health and economic consequences with 110 million primary care visits, 20 million days each of school and work absences, and a total economic impact approaching 40 billion U.S. dollars annually in the U.S. alone.[3,4] In addition, colds also cause morbidity if infections result in health complications, such as pneumonia and exacerbations of asthma, Chronic Obstructive Pulmonary Disease (COPD), and cystic fibrosis. The latest World Health Organisation (WHO) report on the global burden of disease noted complications from respiratory infections resulted in three million deaths world-

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wide, making it one of the leading infectious causes of death in adults and the leading cause of death in children under five.[5]

Historically, transmission studies of colds relied upon experimental infection of index cases with laboratory passaged virus.[6] Challenge studies with respiratory viruses are still undertaken, primarily to assess candidate vaccines, antivirals and host-virus interactions, but they employ virus strains that are manufactured according to Good Manufacturing Practice (GMP) standards required for vaccine manufacture.[7] The manufacturing process requires highly specialised expertise, equipment and facilities and is costly, so comparatively few respiratory virus strains meet the required GMP standards.[7] Research examining the natural transmission of respiratory viruses has been limited. Studies that have attempted to do this have mainly studied transmission within households or offices, were performed using aerosol sampling devices that are not routinely available or were constrained by difficulties in controlling the interactions.[8–10] The deficiencies of such studies pose obstacles in measuring the effect size of interventions and devising strategies to reduce transmission and highlight the need for an infection model that mimics the natural transmission of human respiratory viruses. We aimed to evaluate the feasibility of a novel method to detect human-to-human transmission of respiratory viruses using controlled interactions between healthy adult volunteers and children hospitalised with ARIs.

Methods

Study design and participants

We undertook a prospective study between September 2012 and May 2015 at Leicester Children's Hospital, University Hospitals of Leicester National Health Service (NHS) Trust, Leicester, UK. A workflow diagram showing source and contact recruitment and the study procedures is provided in Supplementary Fig. 1.

We recruited hospitalised children with ARIs as potential index cases. Study specific criteria were: age 6 years or younger, were full-term at birth, had no cardiac or metabolic abnormalities, and presented to hospital with at least two of any respiratory signs or symptoms, with onset within 216 h (9 days) of recruitment. Defined respiratory signs and symptoms were: nasal discharge, sneezing, hoarseness of voice, cough, difficulty in breathing, rapid breathing, chest recession, crackles on auscultation and wheeze. Children were not pre-screened for respiratory viruses. Following consent, we collected demographic information and clinical details regarding eleven signs and symptoms shown in Table 1. For each of the children with ARIs these eleven signs and symptoms were recorded as yes (1) or no (0) and the total number of signs and symptoms was calculated.

We recruited healthy medical students who satisfied inclusion and exclusion criteria as contacts. Study-specific criteria were: no chronic medical conditions, no immune function disorders, aged 18 to 35 years, no symptomatic ARIs during the 14 days before the interaction, afebrile (oral temperature <374 °C) on day 0, and not pregnant. Adult contacts were able to participate in multiple interactions providing it was at least 28 days between interactions, and they also fulfilled all the study-specific criteria above.

Procedures

We collected nose and throat swabs from index cases (children with ARIs) and their adult contacts in separate rooms prior to their controlled interaction. One child and one contact interacted in the controlled setting on each occasion, in the presence of a parent or guardian and a research team member, wearing appropriate personal protective equipment. Interactions occurred in a hospital side room with no active ventilation, with the windows and door closed. A 30 min timed interaction comprised three cycles, each including four minutes of interactive toy-playing, five minutes of clinical examination, and one minute of the contact rubbing their face.

Table 1
Children with ARI and adult contact demographics.

	Children with ARIs (n = 111)	Adult contacts (n = 111*)
Age		
Mean Age (±SD)	26 mos. (18)	22 yrs. (3)
Median Age (IQR)	22 mos. (13–35)	21 yrs. (20–23)
Sex (%)		
Male	67 (60)	50 (45)
Female	44 (40)	61 (55)
Ethnicity (%)		
White	71 (64)	75 (68)
Asian	23 (21)	23 (21)
Other Ethnicity	17 (15)	13 (12)
Signs and Symptoms at Presentation (%)		
Cough	109 (98)	NA
Shortness of Breath	105 (95)	NA
Tachypnoea	97 (87)	NA
Nasal Discharge	94 (85)	NA
Wheeze	94 (85)	NA
Sneezing	80 (72)	NA
Hoarseness	64 (58)	NA
Chest Recession	60 (54)	NA
Temperature >37.8 °C	27 (24)	NA
Crackles on Auscultation	24 (22)	NA
Headache	7 (6)	NA
Total number of signs and symptoms, median (IQR)	8 (7–9)	NA

*80 Adult contacts took part in interactions, with 34 Adult contacts taking part in multiple interactions after a period of 28 days, and only if they satisfy inclusion and exclusion criteria at each stage of the interaction (see Fig. 1). Adult contact demographics shown are from each adult in all 111 interactions.

Follow-up required contacts to return on four occasions for nose and throat swabs specimen collected on days 1, 3 to 5, 6 to 7, and 8 to 10. Contacts also completed a symptom questionnaire three times daily for ten days, beginning at the first opportunity after the interaction. We used the modified Jackson score to quantify subjective upper respiratory tract symptoms, a commonly used scoring system to assess ARI illness severity in adults.[11,12] On days 0 to 10 inclusive, contacts recorded their subjective ratings of eight symptoms, specifically nasal congestion, rhinitis, sneezing, cough, sore throat, malaise, headache or chills. The eight symptoms were scored as 0 = none, 1 = mild with no limitations to normal activity, 2 = moderate and some limitation to normal activity, 3 = severe without needing medical attention, and 4 = incapacitating and needing medical consultation. A total score was calculated for each day from which the total score for day 0 was subtracted to compute an adjusted score for each day. The mean of the five highest consecutive adjusted daily scores was then calculated to give an overall modified Jackson score.[11] We used a mean score of >6 as indicative of a clinical cold to evaluate symptoms in relation to the Reverse Transcription Polymerase Chain Reaction (RT-PCR) findings.[11]

Ethics

This study was approved by the Derby National Research Ethics Service, UK (REC number 12/EM/0341). Approval was also given by the University of Leicester Medical School for the involvement of medical students in the study. Medical students were recruited following attendance at study information sessions; recruitment was not linked to medical school attainment in any form. Study information sessions were conducted by a member of the study

team and were independent of the medical school. Paediatric guardians and adult volunteers provided written informed consent in accordance with the Declaration of Helsinki and Good Clinical Practice in Research.

Laboratory procedures

We performed RT-PCR on all nose and throat swabs as previously described.[13] Quantitative RT-PCR was used to determine viral load for EV, Flu, RSV, and RV. For contacts that were Polymerase Chain Reaction (PCR) positive at multiple follow-up swabs, the highest viral load from all swabs was recorded as the peak viral load. Qualitative PCR was used to detect AdV, CoV, HMPV and PIV, and for detection of the human Ribonuclease P gene to verify correct nose and throat sampling.

Eurofins Genomics, UK did the sequencing of specimens collected from adult contacts on Day 1–10 who were also RT-PCR positive on Day 0. The corresponding sample from the child with ARI that interacted with this adult was sequenced. In addition, all other RV-positive samples from children with ARI and contacts were sequenced to determine RV types. The Basic Local Alignment Search Tool was used to ascertain the similarity between the sequences of virus from children with ARI and their paired contact. We classified a sequence homology of 90% or more as detection of the same virus.[14–16]

Outcomes

We defined virus transmission by the occurrence of at least one of the two following conditions: (1) Contact's swabs were RT-PCR negative immediately before the interaction but became RT-PCR-positive within 10 days of the interaction with the same viral species as identified in the paired child, (2) The contact was RT-PCR positive for the same virus as the child immediately before the interaction and sequencing revealed <90% homology between Day 0 specimens of both, AND swabs collected from the contact during the 10 days after the interaction revealed ≥90% sequence homology as the child's Day 0 specimen.

We defined an infection without a clinical cold in the adult contacts if they fulfilled the criteria above and had an overall modified Jackson score of <6.

We selected variables for the multivariable model that might be associated with viral transmission based on existing literature and expert opinion. The covariables used are listed below:

- Demographic characteristics of contacts and children with ARIs (Age, sex, ethnicity).
- Illness factors for children with ARIs (symptom duration, total number of signs and symptoms, presence of cough, presence of sneezing, peak viral load, PCR positivity for rhinovirus, PCR positive for multiple or a single respiratory virus).
- Duration of interaction.

Statistical analysis

The demographic characteristics of the cohort were described using median and interquartile range (IQR) (for continuous variables) and proportions/percentages (for categorical variables). Comparisons were made using the non-parametric Mann-Whitney U test and Pearson's chi-squared test (or Fisher's exact test, if appropriate), respectively. Cycle threshold (Ct) values were used to make comparisons of viral loads in positive PCR swabs of Children with ARIs. Logistic regression was used to derive unadjusted and adjusted odds ratios describing the relation between covariates and the occurrence of a transmission event. Because some adult contacts took part in more than one interaction we completed a sensitivity analysis using only adults that took part in one interaction to ensure that the relation between covariates and the occurrence of transmission remained. Statistical computations used Microsoft Excel (version 2010), open-source statistical software (www.openepi.com) and Stata (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC.). p values ≤0.05 were considered statistically significant.

Results

Over the study period, we recruited 154 paediatric inpatients to participate as index cases and 191 healthy adults as contacts; recruitment is summarised in Fig. 1. Demographics of the children and adult contacts who took part in an interaction are shown in Table 1, along with the clinical features at presentation for the children with ARIs. Interactions commenced at a median of 60 min (range 20–180 min) following consent by a child's parent or guardian and lasted for a median of 25 min (IQR 20–30 min). Following all 111 interactions, all adult contacts completed and returned diary cards and they attended 552 (99%) of 555 scheduled appointments.

Of the 111 interactions that were completed, 103 children with ARIs had a positive RT-PCR of nose and throat swabs taken immediately before the interactions, 58 (56%) children had one virus detected, 35 (34%) had two viruses detected, and 10 (10%) had three viruses detected. The viruses detected from the children with ARIs and viral loads are presented in Table 2. 61 RVs were speciated: RV-C was the most common ($n = 38$), mostly as co-infections ($n = 33$), followed by RV-A ($n = 19$, with seven co-infections), and then RV-B ($n = 4$, with all four as co-infections).

Fig. 2 shows the transmission events determined by RT-PCR (supported by sequencing in three cases) of nasopharyngeal specimens from the 103 contacts of RT-PCR-positive children. The overall virus transmission rate was 15% (15/103 interactions). RVs were transmitted during 14 interactions, and RSV-B was transmitted in a single case, giving transmission rates of 21% (14/67) for RV, 5% for RSV-B (1 of 21), and 4% (1/28) when RSV-A and RSV-B groups are aggregated.

Table 3 shows the results of the univariable and multivariable analyses of children with ARI and contact demographic and clinical factors associated with the 15 interactions that resulted in respiratory virus transmission. On multivariable analysis, transmission was associated with child positivity for rhinovirus (adjusted odds ratio (aOR) 18·8 (95% confidence interval 1·64–214·3) and inversely associated with the contact being male (aOR 0·12 (95% confidence interval 0·02–0·72). Amongst those interactions where only rhinovirus was transmitted, on multivariable analysis the same variables were associated with transmission (Table 3). On a sensitivity analysis using only adults that took part in one interaction, transmission events were again more common in females compared to males (6 transmission events Vs 2 transmission events)

Amongst the 103 adult contacts, the median modified Jackson score was 0.6 (IQR 0–1·6). Of the 15 transmission events, 13 resulted in infection without a clinical cold (a modified Jackson score of <6) in the adult contacts. Symptom scores did not vary significantly between contacts where transmission occurred and those where transmission did not occur (Transmission median 0·8 IQR 0–0·34 vs No transmission median 0·6 IQR 0–1·4, $p = 0·21$). Four contacts (4%; 4/103) met the study criteria for a clinical cold (a modified Jackson score of ≥6). Two of these four contacts were PCR-negative throughout the study follow-up period. Two contacts reported symptoms with a severity score of 4 (incapacitating and needing medical consultation, but at only one timepoint for both contacts. One contact experienced a severe sore throat and tested positive for RV. The other contact reported a severe fever but was PCR-negative throughout the follow-up period.

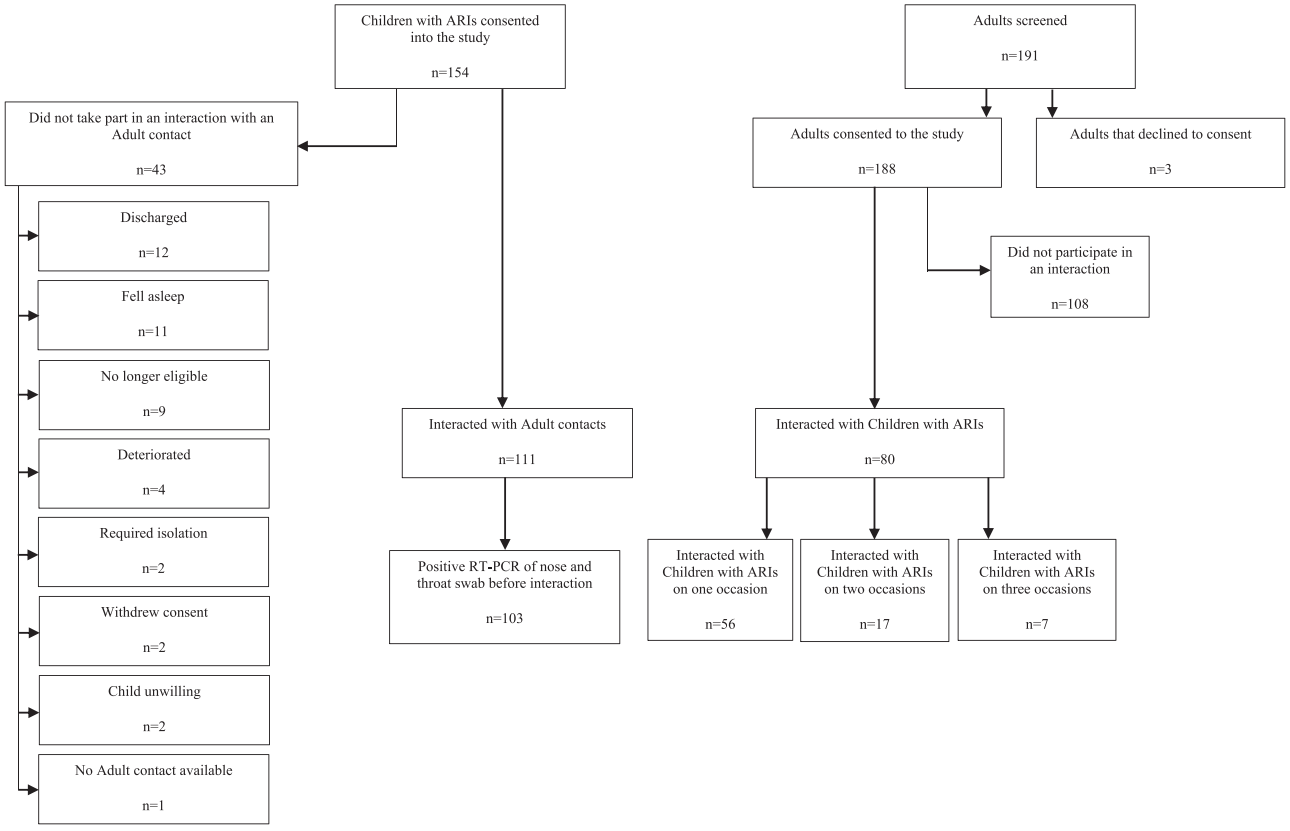


Fig. 1. Flowchart of recruitment of Children with ARIs and Adult contacts.

Table 2
Viruses Detected by PCR in the Nose or Throat of Children with ARIs $n = 103$ and Adult Contacts $n = 103$.

Virus[a]	PCR-positive children with ARIs (%) $n = 103$[b]	Median cycle threshold for positive children with ARIs (IQR)	Contacts where transmission detected (%) $n = 15$	Transmission probability^ (%)
RV	67 (65)	30.21 (27.7–32.9)	14 (93)	14/67 (21)
EV	26 (25)	34.10 (30.85–38.02)	0	0
RSV-B	21 (20)	24.89 (20.66–28.58)	1 (7)	1/21 (5)
AdV	11 (11)	26.57 (22.8–29.07)	0	0
PIV	10 (10)	25.35 (23.4–26.57)	0	0
RSV-A	7 (7)	23.74 (17.6–30.42)	0	0
HMPV	7 (7)	29.31 (24.34–32.11)	0	0
FluA	4 (4)	24.05 (22.26–26.57)	0	0
CoV	5(5)	27.7 (24.62–29.07)	0	0

[a]RV, rhinovirus; EV, enterovirus; RSV, respiratory syncytial virus; AdV, adenovirus; PIV, parainfluenzavirus; HMPV, human metapneumovirus; Flu, influenza; CoV, coronavirus.
[b]n = number of samples in which at least one virus was detected by PCR is 103. N.b the total number of viruses detected is greater than 103 since 45 Children with ARIs were positive for >1 one virus.
^Transmission probability equals the number of contacts where transmission was detected for a particular virus divided by the total number of interactions with a child positive for that same virus.

Discussion

We completed a prospective study to evaluate the transmission rate of respiratory viruses in a healthcare setting during 30-minute controlled interactions that simulate clinical encounters between children and healthcare personnel. We selected young children with ARIs as index patients because they suffer more ARIs than older age groups, have higher rates of respiratory virus positivity than older children and adults, and suffer high hospitalisation rates for respiratory disorders.[2,17,18] We used medical students as contacts because of their knowledge, general interest, skills, and likely acceptability to parents of children with ARIs. The interactions were designed to provide a controlled evaluation of nosocomial transmission of respiratory viruses in hospitals, and a possible platform for multicentre studies.

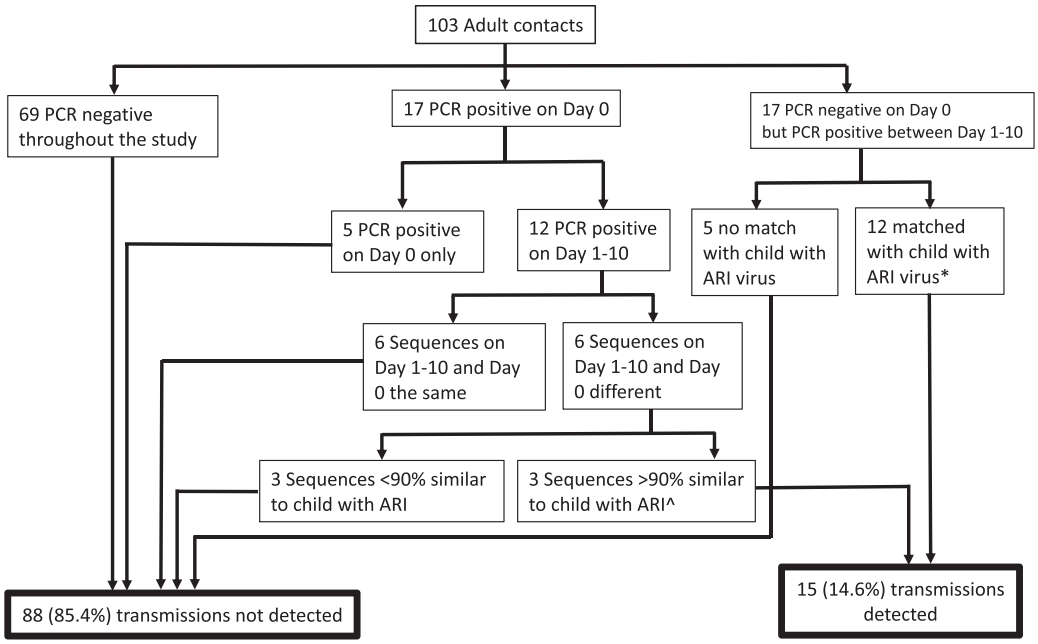
Altogether 93% of children with ARIs shed one or more respiratory viruses, mostly RVs, consistent with previous studies and the many serotypes of RV (>160) that display little cross-reactivity to neutralising antibodies.[2,18] The children with ARIs tolerated the interactions well, as assessed by the median duration of 25 min and the paired contacts were highly motivated as illustrated by their compliance with diary completion and attendance at follow-up appointments.

The transmission rate for RVs during the 30 min interactions was 21%. The RV transmission efficiency compares with the observed rate between RV-infected children (median age 1·3 years)

Table 3
Characteristic of Participants in the Transmission Events Determined by PCR/sequencing.

Variable	Transmission events (all) 15/103 (%)	Unadjusted OR (95% CI)	Adjusted OR[1] (95% CI)	p	Transmission events (rhinovirus only) 14/67	Unadjusted OR (95% CI)	Adjusted OR[2] (95% CI)	p
Contact age		0.97 (0.79–1.20)	0.86 (0.65–1.14)	0.29		0.95 (0.78–1.17)	0.86 (0.64–1.15)	0.25
Contact sex								
Female	11/58 (18.9)	1	1	0.02	10/38 (26.3)	1	1	0.03
Male	4/45 (8.9)	0.42 (0.12–1.41)	0.12 (0.02–0.72)		4/29 (13.8)	0.48 (0.12–1.61)	0.07 (0.01–0.72)	
Contact ethnicity								
White	9/69 (13.0)	1	1	0.58	8/42 (19.1)	1	1	0.87
Asian	4/22 (18.2)	1.48 (0.41–5.38)	3.42 (0.53–22.0)		4/16 (25.0)	1.42 (0.36–5.57)	8.07 (0.74–88.4)	
Afro-Caribbean/Other	2/12 (16.7)	1.33 (0.25–7.09)	1.33 (0.14–9.35)		2/7 (22.2)	1.21 (0.21–6.99)	1.64 (0.15–17.6)	
Children with ARI age		1.02 (0.99–1.06)	1.03 (0.99–1.07)	0.17		1.02 (0.99–1.05)	1.05 (0.99–1.07)	0.11
Children with ARI sex								
Female	4/42 (9.5)	1	1	0.25	3/24 (12.5)	1	1	0.11
Male	11/61 (18.0)	2.09 (0.62–7.07)	2.64 (0.50–13.9)		11/43 (25.6)	2.41 (0.60–9.66)	5.39 (0.70–41.8)	
Children with ARI ethnicity								
White	8/63 (12.7)	1	1	0.24	7/41 (17.1)	1	1	0.12
Asian	2/23 (8.7)	0.65 (0.13–3.34)	0.25 (0.03–2.02)		2/15 (13.3)	0.75 (0.14–4.08)	0.34 (0.04–3.16)	
Afro-Caribbean/Other	5/17 (29.4)	2.86 (0.80–10.3)	2.14 (0.03–14.0)		5/11 (45.5)	4.05 (0.96–17.1)	8.74 (0.82–93.11)	
Duration between children with ARI illness onset and interaction (hours)		1.01 (0.99–1.03)	1.03 (0.99–1.07)	0.03		1.02 (0.99–1.04)	1.05 (1.00–1.09)	0.02
Duration of interaction (minutes)		1.04 (0.96–1.14)	0.99 (0.89–1.10)	0.90		1.02 (0.93–1.11)	0.96 (0.86–1.08)	0.52
Children with ARI Total number of signs and symptoms		1.00 (0.73–1.38)	1.10 (0.70–1.72)	0.69		1.25 (0.85–1.85)	1.35 (0.75–2.44)	0.32
Children with sneeze	11/77 (14.2)	0.92 (0.26–3.17)	0.57 (0.09–3.70)	0.55	10/45 (22.2)	1.28 (0.35–4.68)	0.23 (0.03–2.03)	0.19
Children with cough	15/102 (14.7)	*	*		14/66 (21.2)	*	*	
Source swab positive for rhinovirus								
No	1/36 (2.8)	1	1	0.02				
Yes	14/67 (20.0)	9.25 (1.16–73.5)	18.8 (1.64–214.3)					
Children swab positive for >1 virus								
No	8/58 (13.8)	1	1		7/29 (24.1)	1	1	
Yes	7/45 (15.6)	0.91 (0.30–2.73)	1.58 (0.32–7.95)	0.576	7/38 (18.4)	0.71 (0.22–2.31)	0.99 (0.17–5.89)	0.99
Children with ARI viral load						1.00 (0.99–1.00)	1.00 (0.99–1.00)	0.70

OR, Odds ratio; CI, Confidence interval; [1]Model included adjusted variables: Contact & Children with ARI age, sex, ethnicity, Duration between children with ARI illness onset and interaction, duration of interaction, children with ARI total number of signs and symptoms, presence of sneeze and cough in child with ARI, children with ARI swab positive for rhinovirus and Child PCR positive for single or multiple respiratory viruses prior to the interaction; [2]Model included adjusted variables: Contact & Children with ARI age, sex, ethnicity, Duration between children with ARI illness onset and interaction, duration of interaction, children with ARI total number of signs and symptoms, presence of sneeze and cough in child with ARI, Child PCR positive for single or multiple respiratory viruses prior to the interaction and peak viral load; * Cough was present in all except one child with ARI so was omitted as predictor of a transmission event.



ARI, Acute viral Respiratory infection; * Contact's swab became RT-PCR-positive with the same viral species as identified in the paired child with ARI;
^ Contact was RT-PCR positive for the same virus as the child immediately before the interaction and sequencing revealed <90% homology between Day 0 specimens of both, AND swabs collected from the contact during the 10 days after the interaction revealed ≥90% sequence homology as the child's Day 0 specimen

Fig. 2. Flowchart of respiratory virus transmission events in Adult contacts that took part in interactions with a PCR positive child with ARI n = 103.

and their parents (7 of 14 infected, 50%) in households when adjusted by the 'background' incidence of RV infections in parents (7 of 30 infected, 23%) of RV RT-PCR-negative symptomatic children.[19] Contact between the index cases and parents in this household study would have been more frequent, intimate, and prolonged than in our study, but household studies do not provide this level of information. Experimentally induced RV infection in human volunteers employs laboratory grown virus and pre-screening of participants and contacts for absence of neutralising antibody to increase the likelihood of infection in index cases and their contacts. Transmission of laboratory strains of RV in such studies is reported as unusual and requires donors and recipients to spend many hours together. One study of married couples reported a transmission rate of 41% with contact periods of 63–149 h.[20] Another reported the aerosol transmission of RV in 56% of contacts during interactions lasting 12 h.[21] The RV transmission rate in our study is substantially lower than the infection rate of ~90% seen currently after viral challenge of serologically-susceptible subjects.[7] It is questionable, however, whether the limited number of laboratory-grown RVs are appropriate surrogates for >160 distinct RV serotypes. As an example, RV-C species are associated with a greater severity of asthma exacerbations in children and a higher rate of pneumonia in adults than with RV-A infections, suggesting differing immunopathogenesis by species and possibly serotype.[18,22]

Our study identified a higher virus transmission rate in women than in men. This is in keeping with the higher rates of ARIs observed in females in household studies of ARIs.[23,24] The differences by sex in those studies occurred irrespective of the presence of household children.[24] Two further studies, in Denmark and Australia, – noted similar trends by sex.[25,26] Our study, which used a standardised interaction, is the first demonstrating a sex difference for transmission of RV. A study supervisor was present throughout all interactions, ensuring that there was no effect of gender on the actual conduct of the interactions. The increased susceptibility to infection by RV in women contrasts with observed sex differences in the pathogenesis and severity of viral respiratory infections, in which males tend to be more vulnerable to severe outcomes.[27]

Virus transmission in our study was defined by RT-PCR and sequencing while symptoms and symptom scores assessed safety and tolerability of the interactions. The after-effects of the interactions were well tolerated by contacts, as assessed by non-significant differences between the symptom scores of contacts in whom transmission did and did not occur, and few reports of modified Jackson scores of >6 and severity scores of 4. Symptom scores were extremely poor in identifying virus transmission. Two contacts with symptom scores of >6 remained RT-PCR negative. This may be due to infection by a virus not included in the multiplex RT-PCR, or the tendency for adults to shed lower titres of respiratory viruses than young children.[28] Conversely, only two of 14 contacts in whom RV transmission occurred reported modified Jackson scores of >6. The similarity of symptom scores in infected and uninfected contacts is in keeping with other studies using RT-PCR, including a four-fold higher incidence of asymptomatic RV infections than symptomatic RV infections among university students, and high rates (65–97%) of asymptomatic ARIs in an ambulatory population.[29,30] These observations have important implications for the use of personal protective equipment (PPE) and the design and ventilation of institutions providing care to vulnerable patients.

This study achieved its goal in transmitting respiratory virus, mostly RV, from children to adults using short, standardised interactions. Nonetheless, there is room for modification. First, we focused on virus transmissions rather than the means of transmission. Participants could, for example, be screened during interactions or fomites screened. Air sampling is another possibility. Second, apart from RV and RSV-B, transmission of other viruses was not detected. This is possibly because many children had co-infections (45%), raising the possibility of interference between co-infecting viruses, leading to decreased shedding of one or more viruses.[17] In addition, we used a 90% sequence homology threshold to identify three cases of RV transmission. Sequencing allowed us to identify additional transmission events, however there is a

possibility that in this study we have overestimated transmission events and in future work, where we evaluate the transmission rate as a primary outcome, we would look to use a higher cut off to confirm transmission. Had we excluded children with RV infections or co-infections, after a rapid point of care screening test, transmission of other viruses may have been seen. Third, some adult contacts took part in multiple interactions which may have introduced bias in the results. To overcome this a minimum 28-day gap between interactions ensured adults contacts were functionally independent. Analysis of the data retrospectively showed transmission events were evenly distributed between those that took part in multiple and just one interaction giving support to our assumption that contacts were independent. Fourth, the difference in transmission rates for RVs compared to all other viruses suggest that viral, host or environmental factors are important determinants of virus transmission. For example, evidence indicates that as non-enveloped viruses RVs are more stable on contaminated surfaces than enveloped viruses, like influenza and RSV, and possibly more transmissible as a result.[31] Evaluation of the factors that influence successful transmission was beyond the scope of this preliminary study. The standardised methods used in our study should provide the means to resolve such questions and permit comparison of transmission rates and determinants of transmission by different viruses and in different settings such as in the community.

In summary, our study resulted in three key findings. First, relatively brief standardised interactions in a hospital setting resulted in natural transmission of RVs and RSV-B that were circulating locally. Second, we found that using this methodology transmission events were more common when children were positive for RV. And third, we showed the effect of sex on virus transmission (higher among women). Our method offers some key advantages in comparison to virus challenge methods, including its potential use in healthcare settings.

Declarations

Ethics approval: This study was approved by the Derby National Research Ethics Service, UK (REC number 12/EM/0341). Approval was also given by the University of Leicester Medical School for the inclusion of medical students to partake in the study. Paediatric guardians and adult volunteers provided written informed consent in accordance with the Declaration of Helsinki and Good Clinical Practice in Research.

CRediT authorship contribution statement

Marie-jo Medina: Conceptualization, Data curation, Formal analysis, Project administration, Writing – review & editing. **Joshua Nazareth:** Data curation, Investigation, Writing – original draft. **Helen M. Dillon:** Conceptualization, Data curation, Writing – review & editing. **Christopher J. Wighton:** Visualization, Data curation. **Srini Bandi:** Visualization, Data curation. **Daniel Pan:** Writing – review & editing. **Karl G. Nicholson:** Conceptualization, Visualization, Data curation, Methodology, Writing – review & editing. **Tristan W. Clark:** Conceptualization, Visualization, Writing – review & editing. **Peter W. Andrew:** Conceptualization, Visualization, Data curation, Writing – review & editing. **Manish Pareek:** Conceptualization, Visualization, Formal analysis, Writing – review & editing.

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Data sharing

All data relevant to the study are included in the article or uploaded as supplementary information.

Declaration of Competing Interest

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2022.08.004.

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