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State of the Science Review

Transmission route of rhinovirus - the causative agent for common cold. A systematic review

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A B S T R A C T

Background: Human rhinoviruses (RVs) are the most common cause of acute respiratory tract illness and upper respiratory tract infections, traditionally defined as 'common colds'. Experimental transmission of RV has been studied for more than 50 years. However, there are divergent results as to whether hands and fomites or aerosols constitute the dominant route of transmission in natural settings.
Methods: We have systematically reviewed the literature according to the PRISMA 2020 statement. Searches were run in PubMed and Web of Science until August 2022. Inclusion criteria were original studies of relevance for revealing the route of transmission of rhinovirus in humans.
Results: The search yielded 663 results, and 25 studies met the inclusion criteria and were selected for this review. These articles addressing RV transmission routes were assigned to 1 of 3 groups: (1) indirect transmission by fomites and hands, (2) direct transmission via large aerosols (droplets) or small aerosols, or (3) transmission either direct via large aerosols (droplets) or small aerosols and fomite or hands.
Conclusions: We found low evidence, that transmission via hands and fomite followed by self-inoculation is the dominant transmission route in real-life indoor settings. We found moderate evidence, that airborne transmission either via large aerosols or small aerosols is the major transmission route of rhinovirus transmission in real-life indoor settings. This suggests that the major transmission route of RVs in many indoor settings is through the air (airborne transmission).

BACKGROUND

Acute upper respiratory viral infections, the clinical syndrome we refer to as the common cold, is the most frequent acute respiratory illness experienced by humans. Adults will experience 2-4 colds each year, while children experience 6-10. Although common colds can be caused by a variety of different viruses, including coronaviruses, parainfluenza virus, and respiratory syncytial virus, the predominant viral pathogens are rhinoviruses (RV).[1,2]

Rhinoviruses present a considerable burden to public health and are an important cause of work absenteeism in many jobs.[3] Knowledge of virus transmission and prevention measures is crucial for a cost-effective prioritization of mitigating efforts within both the working environment, health care facilities, and society. Efforts at vaccine development are hindered by the existence of more than 100 RV serotypes with large variability in the antigenic sites.[4]

Rhinoviral infections are spread from person to person by virus-containing respiratory secretions, which may gain entry to a susceptible host's respiratory tract. Respiratory viruses can spread from one infected person to another via 3 different transmission routes: contact (direct or indirect), large aerosols (often referred to as droplets), and smaller aerosols. Direct contact can be through touching, kissing, or indirectly through hands or intermediate objects (fomites). The larger aerosols, which fate in the air is determined mainly by gravity will, in a matter of milliseconds, settle on surfaces, and their infectivity is influenced by the survival of the virus on surfaces and the subsequent transfer to a susceptible host.[5] The small aerosols can remain

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airborne for a long time, at least hours, and transfer over longer distances indoors and be inhaled by a susceptible host.[6,7]

An extensive amount of new evidence has emerged in the corona era, which shows that airborne infection is the primary route of transmission for SARS-CoV-19 and probably other respiratory viruses.[7-10] The fate of larger aerosol particles for example, generated during coughing, sneezing, and talking is determined by gravity and air currents; strong flows can keep particles as big as 100 μm afloat, where as in still air they might otherwise settle out due to gravity more quickly. Often these larger aerosols will evaporate leaving a much smaller particle behind, better capable of remaining airborne,[11] and new research demonstrates, that infectious aerosols are generated through talking, singing, and breathing.[12-15] A question for many years is which transmission route is the dominant one for the rhinoviruses.

It is well known, that deposition of rhinovirus directly on the nasal mucosa or conjunctiva[16] or nasal inhalation of small aerosols (0, 2-3 μm)[17] will induce a disease similar to the common cold in 60%-100% of the cases. However, the relative contribution of each mode to the transmission of a particular virus in different settings, and how its variation affects transmissibility and transmission dynamics is not clear. In a review article back in 1978, Gwaltney and Hendley, 2 of the key researchers in the field, wrote: "It can rightfully be asked whether these experimental findings have any relation to spread of rhinovirus colds under natural conditions. The answer to this question is uncertain...".[18] Hence, the ambition of the present review was to determine if there is new and reliable knowledge that can help to identify which route of infection is most important for rhinovirus and common colds in natural settings.

Rhinovirus

In addition to common cold, RVs are the primary causes of exacerbation of chronic obstructive pulmonary disease and asthma and generate more consultations annually than any other viral or bacterial source of respiratory illness.[19-22] As a result, RVs are associated with significant burdens in terms of medical visits as well as work and school absenteeism. The clinical presentation of RV infections is varied and ranges from mild upper respiratory tract illnesses, common cold, to more severe lower respiratory tract illnesses. Common cold is defined as an acute upper respiratory tract infection with symptoms lasting less than 10 days. Symptoms typically include nasal discharge, congestion, sore throat, cough, general malaise, and headache.[23] RV infections also contribute significantly to otitis media, sinusitis, and lower respiratory illnesses.[24] Frequent RV infections with wheezing during the first years of life can lead to the development of chronic respiratory illnesses such as asthma.[25]

The RVs belong to the *Enterovirus* genus of the family of *Picornaviridae*. The virus is small (≈30 nm) and without a lipid envelope. The genome of the RVs consists of a linear, single-stranded, positive-sense RNA molecule of about 7.2 Kbp. RV binds primarily to receptors on the epithelium cells in the airway; either the intercellular adhesion molecule 1 (ICAM-1) or for RV-C the cadherin-related family member 3 (CDHR3), a protein expressed by ciliated airway epithelial cells.[26,27]

RV shedding is commonly limited to an 11-21 day period in immunocompetent subjects. Adults and children are usually asymptomatic by the fifth day postinfection. A study has determined the asymptomatic shedding of respiratory virus among adults in New York. They found that RVs were the most abundant virus found among asymptomatic adults (half of all positive samples), and in particular during summer months.[28] Similarly in children, it was found, that the most frequent virus (71%) was RV.[29] Asymptomatic RV cariage has been estimated to be 14%-22%.[30,31]

Transmission

Knowledge of transmission routes is vital in mitigating infectious spread, and for the common cold, the potential for disease prevention and saving resources at both individual and societal level is enormous. Different routes, including direct contact via hands and fomites followed by self-inoculation, and via the air as large and small aerosols, may transmit the virus. Experimentally induced rhinovirus infection in human volunteers can be transmitted both via the aerosol route and by hand contact, but for more than 50 years it has been debated what the main route of transmission is in natural settings.[32,33] In this review, we have systematically reviewed relevant studies on transmission of rhinovirus, including possible interruption of transmission, and evaluated the evidence for the different transmission routes.

METHODS

This systematic review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement[34] when appropriate.

Search strategy, screening process, and inclusion criteria

A literature search was conducted for rhinovirus in combination with transmission of the virus as search criteria in PubMed and Web of Science limiting the results to non-review articles in English or Danish. The search string was: *(rhinovirus[tiab] OR "rhino virus"[tiab] OR "common cold"[tiab]) AND (transmission OR infection OR spread) AND (aerosol OR droplet OR fomite OR surface OR surfaces OR hand OR hands) NOT (Review[Publication Type])*. Further, additional papers were identified based on the reference list of relevant papers including review articles. The databases were last searched August 2022.

Inclusion criteria were original studies of relevance for revealing the route of transmission of rhinovirus in humans. Exclusion criteria were review articles, articles if only about infection mechanisms, virus survival in air and on surfaces, molecular biology studies, pharmaceutical development, clinical studies, studies on for example, nasal secretions without virus identification, commentaries, editorials, studies in animals, and mathematical models. The primary outcomes of interest were studies uncovering the major transmission route of rhinovirus in humans either by monitoring transmission with the outcome: disease or viral shedding, or to interrupt a specific transmission route.

The included studies are assigned to 1 of 3 groups for the syntheses: (1) indirect transmission by fomites and hands, (2) direct transmission via large aerosols (droplets) or small aerosols, or (3) both transmission either direct via large aerosols (droplets) or small aerosols and fomite or hands.

Two reviewers (AMM and LA) independently identified eligible articles by screening titles and abstracts and reviewing full-text articles.

Assessment of bias

One reviewer (LA) assessed the risk of bias and extracted data from the included studies, and a second reviewer (AMM) independently checked these. Any disagreements were resolved through discussion. Because of substantial heterogeneity both in study types and time, we assessed risk of bias based on 3 key domains: selection bias, information bias, and confounding bias.[35] Risk of bias for each domain is classified into 4 categories: low (study is comparable to a well-performed randomized trial), moderate (study is sound for a non-randomized study but cannot be considered comparable to a well-performed randomized trial), serious (the study has some important problems), and critical (study is too problematic, no controls or too few observations, to provide useful evidence on transmission route). We present the results in tabular format.

RESULTS

The database search identified 842 records. These were supplemented with 47 articles identified via references in relevant articles. After removal of duplicates, 663 titles and abstracts were reviewed based on inclusion and exclusion criteria and 126 articles remained. The resulting 126 articles were read by both authors and consensus on the inclusion of 25 articles was achieved. The selection of eligible studies is shown in the PRISMA flow diagram (Fig 1). The included studies are shown in Table 1 with central information on design and results relevant for rhinoviral transmission.

The studies applying a mathematical model to provide information on transmission route[57-59] or a surrogate for RV (MS-2 coliphage)[60] were excluded.

In the identified studies, different approaches were applied to substantiate the documentation for the different transmission routes. These approaches include: (1) focus on the direct spread of disease in various settings, designed to identify the major mode of virus transfer, (2) studies trying to interrupt rhinovirus transmission to make probable, that this route is the dominant one, and (3) the survival and transfer of the virus for example, survival on surfaces or in the air or the transfer from surfaces to fingers and further transfer to the nose. The last types of research can be considered as more indirect evidence supporting a specific transfer mechanism and are only included if measures of related illness were investigated. Often articles comprise elements of several lines of evidence.

In Table 1, the eligible articles are organized into 3 groups according to whether they focus on contact transmission, airborne transmission, or both.

We assessed the risk of bias based on 3 key domains: selection bias, information bias, and confounding.[34] The assessment of the studies' risk of bias is shown in Table 2.

For the synthesis of results, the individual studies were grouped depending on whether the focus was on contact or airborne transmission. Most of the studies are relatively small. A supplemental table provides an overview of the size of the intervention and experimental investigations (Supplementary Table S1).

Contact - studies addressing transmission via fomites, hands, and kiss

This route of transmission involves 3 steps: first, contamination of the hands with nasal secretions by the infected individual; second, hand contact between the infected individual and a new susceptible host; and third, self-inoculation of the nose or eyes of the new host by contaminated fingers. Alternatively, surfaces (fomites) may be contaminated by nasal secretions directly from infected hands or by sedimented aerosols, followed by contact from a susceptible host infected via self-inoculation of the nose or eyes of the new host by contaminated fingers.

We identified several studies demonstrating the ability of RV to initiate common cold after inoculation of the nose and eyes.[16,32] All studies display some bias from low to serious (Table 2). Further, it was shown that viral contamination of the fingers is common in the acute stages of colds and that RV can stay infectious over several hours on fomites and skin.[36-38] A large body of experimental data from the same research group demonstrates, that common cold can be mediated via fomites and hands when significant amounts of fresh

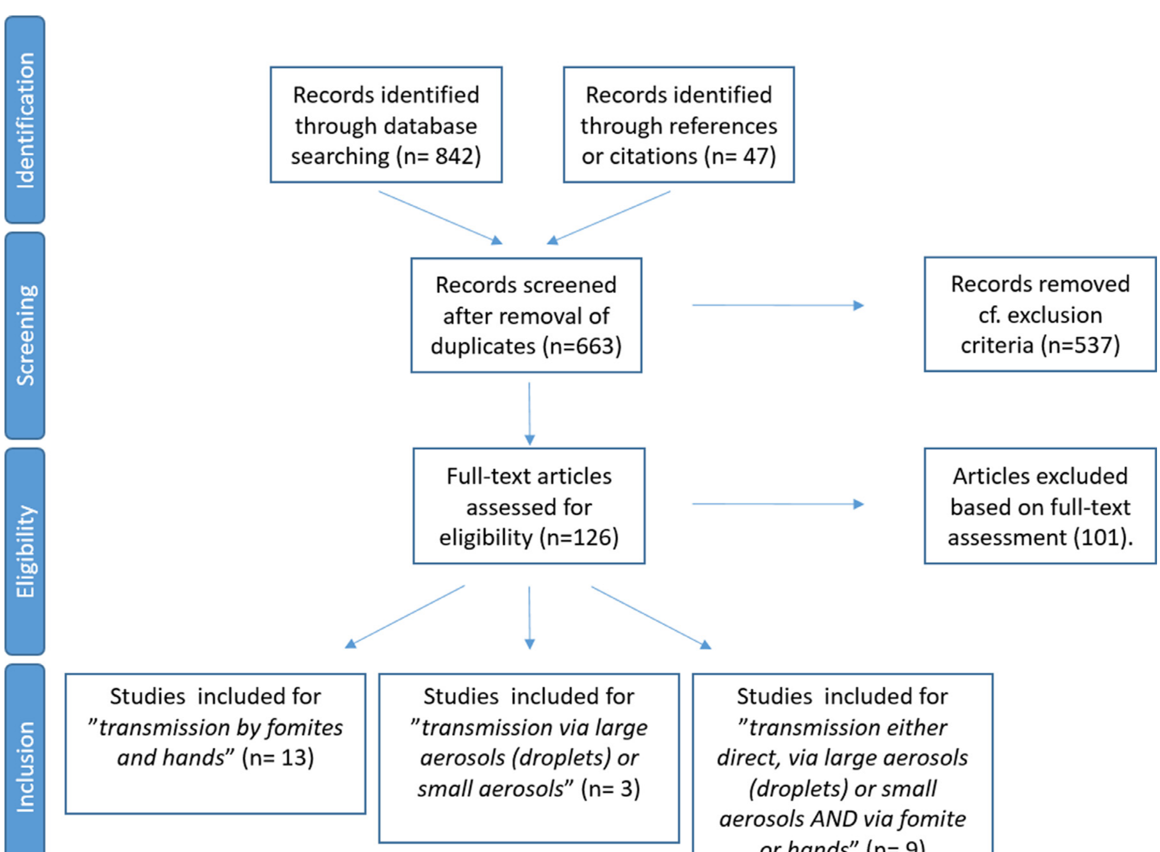

Fig 1. Flow diagram showing the process for inclusion of studies assessing transmission of rhinovirus.

Table 1
An overview of included studies, with focus on design and results relevant for RV transmission

Article:	Study design (from article)	Results	Authors' conclusions
Transmission by fomites and hands			
32	*Laboratory experiments / Human controlled trials* Experimental induction of common cold via contaminated hands and self-inoculation of nose and eyes. Subjects: Employees of an insurance company and members of their family. Exposure / transmission: *Viral shedding:* Samples from nose and throat were tested for RV in 4 persons. The hands of 10 subjects with natural RV colds were tested for the presence of virus. *Virus Survival:* Survival of RV type 39 on skin and surfaces was tested. *Virus transfer:* For another 16 persons the transfer of virus from surfaces to fingers was tested. Self-inoculation of nasal or conjunctival mucosa was examined for 11 volunteers. The subjects rubbed contaminated plastic with his index finger. Four applied the finger to conjunctival mucosa and 7 to the nasal mucosa.	Samples from the nose of 4 persons with natural colds contained RV. Virus was present in the throat of 2 of 4 persons and in the saliva and cough of 1 of 4 persons. Four of 10 subjects with RV infections had virus on their hands. RV survived on plastic and skin up to 3 h (2 h if hands were used), but not from porous material such as facial tissue and cotton cloth. Virus was recovered from fingers in 15 of 16 trials in which plastic previously contaminated, was rubbed by a volunteer. Skin-to-skin transfer was demonstrated in 3 of 4 subjects in which transfer was attempted. Two of 4 contracted RV infection after self-exposure of the conjunctival mucosa to contaminated fingers. Two of 7 became infected after self-exposure of the nasal mucosa.	Dried RV transported on fingers to conjunctival or nasal mucosa initiated infection resulting in a typical common cold in 4 of 11 volunteers. This means of spread may be of major importance in natural RV disease.
36	*Laboratory experiments / Human controlled trials* Possible transmission of RV colds through indirect contact. Subjects: Healthy volunteers aged 18-50, housed in isolation singly or in groups of 2 or 3. *Survival on surfaces:* RVs (RV2) were allowed to dry on marked areas of volunteers' skin, or on smooth surfaces such as stainless-steel, enamel, or plastic table-tops. After the inoculum had dried thoroughly the inoculated areas were sampled. *Transmission of RV between surfaces:* Replicate quantities of RV2 were inoculated on fingers or other areas of skin, or on plastic or steel surfaces, including ball-point pens or spoons. Attempts were made to transfer virus by rubbing firmly for 15 s or, in the case of pens or spoons, by mimicking natural use for 3 min. Exposure / transmission: Comparison of infection via nasal drops, drops into the conjunctiva, or via self-inoculation of volunteers' contaminated forefingers (RV9 was used). Both forefingers were inoculated with virus suspension. Volunteers rubbed each conjunctival fornix for 30 s, or probed each nostril for 30 s.	After 24 h virus was still recovered from the various objects, though at lower titer. When virus was dried on smooth surfaces including table tops, pens and stainless-steel objects, transfer to the recipient finger occurred in 14 of 22 attempts. No transfer was detected in 29 attempts using a dried inoculum on fingers, but some virus could be transferred if the inoculum was not completely dry at the time of contact. RV was recovered from the fingers of 16 of 38 volunteers, who were tested during the acute stages of their colds. When RV from nasal secretions was dried on skin or other surfaces, approximately 40%-99% of infectivity was lost. RV given via nasal drops infected 12 of 15 susceptible volunteers. Two of 4 volunteers were infected when RV9 were dropped directly into the conjunctival fornices. One of 5 volunteers infected themselves by rubbing their conjunctivas with fingers with a dried inoculum of RV9. Two of 6 became infected when the same inoculum was damp when transferred.	Spread of colds is unlikely to occur via objects contaminated by the hands of the virus-shedder, but a recipient might pick up enough virus on his fingers by direct contact with heavily infected skin or secretions to constitute a risk of self-inoculation via the conjunctiva or nostril. The least efficient form of transfer was from contaminated fingers to objects. This could only be achieved if the inoculum was still damp - a circumstance which in daily life would imply very gross contamination. Although the numbers involved in this experiment are small, the results suggest that a relatively large dose of RV9 inoculated indirectly is less efficient in producing infection than a smaller dose given directly into the nose.
37	*Human controlled trials - randomized* Interruption of experimental RV transmission. Subjects: Healthy, young adult volunteers with low or undetectable serum titers of antibody to the challenge RV were recruited from the general population at the University. Exposure / transmission: Volunteers who were "donors" of virus for the hand exposures were challenged intranasally on 3 consecutive days with RV strain HH from the nasal secretions of a young adult with a cold was used. Recipients were randomly assigned to receive iodine or placebo treatment. They immersed each finger and the thumb of both hands in the designated preparation for 5 s. The placebo was an aqueous solution of food colors. An odor of iodine was given to the placebo by adding 0.01% iodine. The donors contaminated their hands with nasal secretions by finger-to-nose contact before the exposure. Hand contact was then made between a donor and a recipient by stroking of the fingers for 10 s. Fifteen minutes after exposure to the donor, the recipients attempted to inoculate their eyes and nose by finger contact with the conjunctival and nasal mucosa. Volunteers were not informed about the contents of the hand preparation until after the study.	None of 8 volunteers became infected when exposed to RV immediately after treatment of the fingers with iodine. All of 7 similarly exposed subjects treated with a placebo preparation became infected. One of 10 volunteers became infected when exposed 2 h after treatment of fingers with iodine, compared with 6 of 10 who became infected in the control group. Virus was recovered from 3 (11%) of 27 hand rinses from volunteers using iodine and from 11 (41%) of 27 hand rinses from volunteers using the placebo preparation.	Aqueous iodine (2%) applied to hands was effective in blocking transmission by hand contact of experimental infection with RV for up to 2 h after application.

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Table 1 (*Continued*)

Article:	Study design (from article)	Results	Authors' conclusions
[38]	*Human controlled trials* Can RV be efficiently transmitted by way of contaminated surfaces? Subjects: Healthy, young adult volunteers were recruited from the community at the University. Exposure / transmission: Donors were inoculated intranasally with RV strain HH. Symptomatic donors deliberately contaminated their hands as in nose blowing and nose wiping. The donors then held the handle of the coffee cup or rubbed the tile with the fingertips of each hand for approximately 5 s. For exposure to the coffee cups, the recipients held and rubbed the handle of the cups as if drinking. For exposure to the tiles, the recipients touched and rubbed the surfaces with the fingertips. After contact with coffee cup or tiles, the recipient placed the fingers in contact with the conjunctival and nasal mucosa as in rubbing the eyes and probing the nose. Outcome: Infection was judged to have occurred if virus was recovered from a nose/throat culture and/or if there was a 4-fold or greater serum neutralizing antibody rise to the challenge virus.	Five of the 10 recipients exposed to contaminated coffee cup handles became infected. Nine (56%) of 16 recipients who contacted contaminated tiles which had not been sprayed with disinfectant became infected. Seven (35%) of 20 recipients became infected after touching tiles which had been sprayed with disinfectant. The efficiency of transmission by the 2 surfaces was similar. Of the recipients who became infected, 62.5% met the criteria for illness. The disinfectant tested in these experiments was moderately effective in eliminating RV from a surface	These studies indicate that experimental RV colds can be spread by way of contaminated environmental surfaces. Two types of contaminated surfaces, ceramic coffee cup handles and plastic tiles transmitted experimental RV infection.
[39]	*Human controlled trials - randomized* To determine whether nasal tissues treated with placebo or virucidal compounds could interrupt hand-to-hand transmission of experimental RV infections. Subjects: Healthy, young adult volunteers were recruited from the community at the University. Exposure / transmission: Volunteers were challenged by a viral inoculum of either RV type 39 or by RV strain HH. Donors blew their nose with 2 hands using either a virucidal tissue (citric acid, malic acid, and sodium lauryl sulfate), a placebo tissue (sodium saccharin), or no tissue (control). The donor then had finger-to-finger contact (10 s) with a recipient. Recipients were exposed to donors on days 3-5, after the donors had developed symptomatic illness. Both donors and recipients wore masks during the contact period. Donors were selected for exposure to recipients only if they had rhinorrhea. Recipients were randomly assigned to the active and placebo tissue and control groups. The recipient was led to another room where self-inoculation (finger contact with the conjunctival and nasal mucosa) was accomplished by rubbing the eyes and probing the nose.	Twenty-four (89%) of the 27 donors had a nose and throat culture positive for RV on at least one of the days of transmission. Virus was detected on the fingers of donors who used no tissue (controls) in 20 (83%) of 24 exposures. Moreover, virus was detected on the fingers of donors using placebo tissues in 26 (42%) of 62 instances and donors using virucidal tissues in 2 (3%) of 62 instances. Infection with RV occurred in 4 (50%) of 8 recipients in the control group, 3 (13%) of 23 in the placebo group, and none of 23 in the active group. The low rate of viral transfer from contaminated fingers of donors who had used placebo tissues to the fingers of recipients was surprising. None of the recipients in the placebo group became infected in the initial first experiment, therefore greater efforts were made to ensure that the tissues were more thoroughly wetted with nasal mucus.	Virucidal tissues are effective in preventing hand contamination during nose blowing. Placebo tissues reduce viral contamination of the hands of the user and also reduce transfer of virus from the user's hands to his contact. Infection rates of recipients in both groups using placebo and active tissues are substantially reduced compared with a control group using no tissues. The conditions of this transmission model prevent extrapolation of these conclusions to the natural setting.
[40]	*Randomized human controlled trials* Interruption of RV transmission via hand route in the home. Subjects: Approximately 50 families (including 473 children) were studied in each of 4 fall periods of 1979-1982. Intervention: The mother began finger treatment with virucidal (iodine) or placebo (randomly) after the appearance of respiratory symptoms in a family member. Finger treatments were made every 3-4 h and after any activities that removed the preparations from the fingers. Surveillance, begun during the first week of September and continued for 5 wks. Illness and RV infection rates were determined in the mother and other family members by daily symptom recording and sampling of respiratory secretions from ill persons. Output: The effectiveness of the finger treatment in protecting against acquisition of colds was determined by calculating secondary attack rates in mothers exposed to an ill family member(s).	The illness rate was 7% (4/58) in the iodine group compared with 20% (16/79) in the placebo group. In each of the 4 study years, mothers in the iodine group had lower rates of illness than did those in the placebo group	The findings of this study support the hypothesis that a proportion of natural common colds is spread by contaminated fingers. There is also the real possibility that a combination of transmission routes are operative in the natural setting. The findings of a reduction in secondary illness rates and an elimination of proven secondary RV infections by the iodine finger treatments support the hypothesis that at least in part the hand contact route is operative under natural conditions.

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Table 1 (*Continued*)

Article:	Study design (from article)	Results	Authors' conclusions
[41]	*Human controlled trials/ intervention – randomized cluster* Preventing transmission of acute upper respiratory infections in children who attend child-care. Subjects: Eligible children were 3 y of age or younger attended the child-care center for at least 3 d/wk. Eleven intervention and 12 control day-care centers comprising 299 children in the intervention arm and 259 children in the control arm were included. Intervention: Consisted of training sessions of good health for child-care workers (3 h long for all staff) and a practical exercise of handwashing and aseptic nose wiping. The intervention took place from March to November (Southern hemisphere). Outcome: The primary outcome measures were parent reports of symptoms of illness in telephone interviews every 2 wks. The symptoms of acute upper respiratory illness elicited from parents were a runny nose, a blocked nose, and cough.	The incidence of episodes of colds per child-year was lower in intervention centers than in control centers. A significant reduction in illness was present in children 24 mo of age and under. Improved compliance with infection control procedures was associated with lower illness but the effect was confined to younger children.	This trial supports the role of direct transmission of colds in young children in child-care. The ability of infection control techniques to reduce episodes of colds in children in child-care was limited to children 24 mo of age and under. To have an impact in young children, infection control techniques needed to be used consistently. Implementing recommended handwashing <70% of the time had no impact on infection and recommended nose wipes needed to be performed at least 97% of the time to reduce infection.
[42]	*Human controlled trials - double-blind, randomized* Prevention of RV transmission by hand treatments with virucid. Subjects: Volunteers were recruited from Winnipeg and surrounding communities for participation. Subjects were required to be in good health and from 18 to 60 y old. Exposure / transmission: Hands of volunteers were contaminated with RV (type 39) at defined times after application of virucidal treatment. The volunteers attempted to self-inoculate by making contact between the fingers and both the conjunctiva and the nasal mucosa with one hand and quantitative viral cultures were done on the other hand. *First study:* Eighty-five volunteers applied either the vehicle (n=31), 3.5% salicylic acid (n=27), or 1% salicylic acid formulated with 3.5% pyroglutamic acid (n=27). Fifteen minutes after application of the test material, the fingertips of each hand were contaminated with RV type 39. *Second Study:* Thirty volunteers were randomly assigned to use the control preparation, and 92 volunteers were assigned to use 4% pyroglutamic acid. After 15 min, 1 h, and 3 h the fingertips of each hand of the volunteers in the active group (30, 30, and 32 respectively) were contaminated with RV type 39.	*First study:* RV infection occurred in 10 of 31 (32%) of the controls and 2 of 27 (7%) of volunteers in both treatment groups. *Second study:* RV infection occurred in 9 of 30 (30%) of the controls and 4 out of 30 (13%) at both the 15-min and 1-h time points and 7 of 32 (22%) at the 3-h time point when treatment was 4% pyroglutamic acid. This hand treatment had significant virucidal activity that persisted for at least 3 h after application. *None of these reductions in infection rate achieved statistical significance.* The study demonstrated that salicylic acid and pyroglutamic acid applied to human skin had anti-rhinoviral activity that was comparable to that of 2% iodine and persisted for at least 3 h.	We have demonstrated that hand treatment reduces hand contamination with RV and can prevent colds under the controlled conditions of the experimental model. Our studies suggest that organic acids commonly used in over-the-counter skin care and cosmetic products have substantial virucidal activity against RV that persists for 2-3 h after application. The results in this experimental model, however, cannot be extrapolated to the natural setting.
[43]	*Human controlled trials - double-blind, randomized intervention* Prevention of RV transmission by hand treatments with virucid. Subjects: Healthy adult volunteers aged >18 y were recruited from the university community. Volunteers were randomized to either an antiviral hand treatment (n=116) or to a no-treatment control group (n=96). Intervention: The trial was done during 9 wks during the fall. The antiviral hand treatment was applied every 3 h while the subjects were awake. The treatment was a lotion containing 62% ethanol, 2% citric acid, and 2% malic acid previously shown to prevent RV infection for up to 4 h after application. Outcome: All volunteers were seen weekly for nasal lavage for detection of RV. The primary endpoint was the number of RV-associated illnesses. All RV-associated illnesses were based on detection of RV and symptoms on at least 3 consecutive days.	Forty-five of the 116 (39%) treated subjects had at least 1 *RV infection* compared with 47 of the 96 (49%) control subjects. Twenty-six (22%) of 116 treated subjects had at least 1 *RV-associated illness* compared with 23 (24%) of 96 subjects in the control group. The hand treatment did not significantly reduce RV infection or RV-related common cold illnesses.	This study found that use of a virucidal hand treatment had no significant impact on the incidence of RV infection or RV-associated illness. The results of our study call into question commonly held assumptions about the route of spread of RV infection and suggest that studies to define the route of spread in different populations in the natural setting are needed.

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Table 1 (*Continued*)

Article:	Study design (from article)	Results	Authors' conclusions
[44]	*Intervention cohort study* Prevention of infectious transmission by hygiene intervention in day-care centers. Subjects: Thirty day-care centers (DCC) comprising 2,349 children (2-6 y of age) in Iceland participated. After a baseline period of 1 y, half of the DCCs were selected for a hygiene intervention that lasted 1.5 y, while the other half received no intervention. The selection of DCCs for the intervention was based on their willingness to comply with the intervention protocol. Intervention: Intervention focused on: (1) Education on the transmission of microbes and the importance of environmental and hand hygiene (study nurse) monthly during the intervention period, for both the staff and the children. (2) liquid soap was used for hand washing. (3) Staff were encouraged to wash their hands often (training and practical exercises were carried out by the study nurse at 6-mo intervals. (4) The staff and children were encouraged to use hand disinfectant (85% ethanol) after hand washing and instead of hand washing when hand washing was not possible. (5) Staff were instructed to use gloves when changing diapers and cleaning children after toileting. (6) Staff were encouraged to use disposable nose wipes for children. (7) Toys were washed and cleaned with soap at least once a month. (8) Furniture, floors, doorknobs, and toilets were cleaned and disinfected at least once a day. Outcome: The illnesses were registered retrospectively for each child (by parents) for every 6-mo season. Colds were defined as a sudden occurrence of nasal congestion or runny nose with at least one other symptom (cough, fever, sneeze, lethargy).	The incidence rates of all illnesses were similar at the intervention and nonintervention DCCs during both the baseline and the intervention periods. No difference was seen in the use of disinfectant, paper towels, liquid soap, or gloves between the intervention and nonintervention DCCs during the baseline period. During the intervention period, the use of disinfectant at the intervention DCCs increased 5-fold compared with the use during the baseline period, the use of paper towels increased 8-fold, the use of liquid soap 1.1-fold, and the use of gloves 1.2-fold. No change in the use of these items was seen at the nonintervention DCCs during the intervention period compared with the baseline period. A good compliance with the study protocols was claimed by 98% regarding hand washing, 89% with regard to the use of disinfectant, and 93% with regard to cleaning toys.	No significant reduction in the adjusted or crude incidence rates of the illnesses was associated with the hygiene intervention, indicating insignificant effects of the intervention. In conclusion, a hygiene intervention at Icelandic DCCs was not effective in reducing respiratory illnesses in preschool children, most likely due to the high standards of baseline hygiene practices at the DCCs.
[45]	*Cluster randomized controlled intervention* Prevention of infectious transmission by hand hygiene intervention in day-care centers. Subjects: Seventy-one day-care centers (DCC) (randomized, and stratified for size and urbanicity) comprising 34 intervention DCCs (278 children) and 35 control DCCs (267 children) over 6 mo (September-April) in The Netherland participated. Intervention: Intervention consisted of 4 components: (1) hand hygiene products were provided free, (2) training about national hand hygiene guidelines (booklet), (3) 2 team training sessions were given aimed at goalsetting and formulating specific hand hygiene improvement activities, and (4) posters and stickers for both caregivers and children were provided as reminders and cues to action. Two groups in each DCC participated in the study. Outcome: Common cold was defined as a blocked or runny nose with at least one of the following symptoms: coughing, sneezing, fever, sore throat, or earache (registered by parents).	The incidence in intervention DCCs at baseline was 9.8 episodes of the common cold per child-year vs 9.2 in control DCCs. During follow-up there were 8.2 episodes of the common cold in intervention DCCs vs 7.4 in control DCCs. Caregivers' hand hygiene compliance were assessed at baseline and follow-up, and found that hand hygiene compliance increased significantly in intervention vs control DCCs.	No evidence for an effect of the intervention (increased hand hygiene) was demonstrated on the incidence of episodes of the common cold.
[46]	*Cluster randomized controlled intervention* Prevention of infectious transmission by increased washing and disinfection of toys and linen in day-care centers. Subjects: Twelve nurseries in Denmark (587 children, age 6 mo-3 y) randomized to intervention and control groups (6 in each), participated. Intervention: The intervention consisted of washing and disinfection of toys and linen every 2 wks for 3 mo by a commercial cleaning company. The intervention took place from January to March. The extent and causes of sickness absence among the children were recorded in both groups before and after introduction of the intervention. Outcome: Absence data and disease patterns were recorded for each child on a daily basis from December 2012 to March (reported by the parents and recorded by the staff). Further, the microbial pathogen load in the nursery environment was measured.	No difference in hygiene standards was found between the control group and the intervention group. No nurseries in either group had a scheduled plan for cleaning toys or washed the toys systematically. Mean absence due to respiratory infections decreased after the intervention in both the intervention and control groups (from 6.8% to 3.4% in the intervention group and from 5.2% to 4.1% in the control group). The presence of respiratory virus DNA/RNA was widespread. The intervention reduced the presence of RV compared with the control group, but the intervention had no effect on sickness absence or disease patterns in the nurseries.	The intervention had no effect on sickness absence or disease patterns in the nurseries. Although cleaning and disinfection of toys every 2 wks can decrease the microbial load in nurseries, it does not appear to reduce sickness absence among nursery children.

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Table 1 (*Continued*)

Article:	Study design (from article)	Results	Authors' conclusions
47	*Explorative observational study* Association of the presence of indicator microorganisms in the environment, with hand hygiene compliance of nurses and the incidence of infection among residents in nursing homes. Subjects: Sixty nursing homes (NHs) in The Netherlands were included in the trial, representing 3,284 beds. Intervention: Multimodal intervention targeted NH policy changes by auditing personal hygiene rules and available hand hygiene materials, and targeted behavior of nurses through e-learning, 3 live lessons, posters, and a photo competition. Observations: Hand hygiene compliance was measured through unobtrusive direct observation. NHs were observed from 8 AM to 12.30 PM in October 2016 (baseline), February 2017 (during the intervention) and May 2017 (after the intervention). In total, 426 nurse observations and 5,200 hand hygiene opportunities were included in this study. Outcome: Environmental surface samples were collected (from October to October the following year) during a hand hygiene intervention in 60 nursing homes. Results were compared with nurses' hand hygiene compliance and the incidence of infection among residents. Staff member (nurse, team leader, or geriatrician) recorded infections of residents weekly over a 7-wk period.	RV (RNA) was the most prevalent pathogen detected (nurses' station: 41%; toilet: 14%; living area: 29%). Generally, more positive RV samples were found at the nurses' station and in the general living area compared with the toilet. No significant associations were found between hand hygiene compliance and the presence of a microorganism. No associations were found between virus contamination and the incidence of disease.	No convincing associations were found between environmental contamination and hand hygiene compliance or the incidence of disease.
Transmission via large aerosols (droplets) or small aerosols:			
17	*Human controlled trials* Induction of common cold via aerosols infect lower respiratory tract. Subjects: Volunteers were 16 healthy adult males from several federal correctional institutions. Volunteers were selected to be free of detectable neutralizing antibody. Exposure / transmission: Eight volunteers were exposed to RV NIH 1734 in aerosols in 3 different doses. The exposure was by nasal inhalation through a facemask attached to an aerosol tube. Aerosols of particle size from 0.2 to 3.0 μm in diameter were used. Two volunteers were given nasal drops and 6 volunteers were inoculated with a serum-inactivated NIH 1734 to serve as a control.	All 8 volunteers exposed to RV containing aerosols developed rhinitis, cough and constitutional symptoms, and nasopharyngeal wash from all 8 volunteers yielded virus during the first week after inoculation. There was no illness and no infection in any of the 6 volunteers inoculated with serum-inactivated NIH 1734. The 2 volunteers given nasal drops developed milder symptoms. These differences in clinical syndromes presumably exist because of infection and injury of tracheobronchial sites following inoculation by means of aerosol.	Following inhalation of aerosols, containing low doses of a RV all 8 volunteers became infected. While conclusive evidence is not yet available, it is reasonable from the foregoing to hypothesize that transmission of infection by means of naturally produced aerosols could lead to the naturally occurring spectrum of RV disease.
48	*Human controlled trials* Possible transmission of RV by airborne aerosol via the tear ducts in humans. Subjects: Healthy employees and students (without serum-neutralizing antibody) at university were invited to participate in the study (age 18-40 y). Exposure / transmission: The study uses an aerosolization technique to expose eyes-only for RV aerosols. Ten participants (5 women and 5 men) were exposed (for 20 min) to RV type 39 and only the eyes were exposed (aerosol size adjusted to 4.7 μm).	None of the 10 participants developed common cold symptoms or carried the RV on their mucous membranes during the 10-d observation period after exposure. Human RV type 39 could not be detected on the mucous membranes of any participant after exposure.	Transocular exposure to an airborne infectious dose of human RV did not lead to infection. Eye protection is much less important than respiratory protection for preventing transmission of this virus.
49	Observational study among randomly selected day-care centers Association between ventilation in day-care centers and sick leave among nursery children. Subjects: Twenty-three day-care centers (DCCs) were randomly recruited in Denmark. Twenty DCCs comprising 635 children (482 aged 0-3 y and 153 > 3 y) were included in this study. Observations / data: Data on child sick leave within an 11-wk period (January-March) were obtained. Underlying causes were registered by teachers at all DCCs. Three proxies of ventilation efficiency were used. Outcome: Correlation between sick leave among nursery children and ventilation.	A statistically significant inverse relationship between the number of sick days and air exchange rate (ACR), with a 12% decrease in number of sick days per hour increase in ACR. The median temperature and RH during measuring period were 22 °C and 27%, respectively, and the median concentration of CO_2 calculated for opening hours was 579 ppm (mean 643 ppm).	This study suggests a relationship between sick leave among nursery children and ventilation in DCCs. We consider our finding on ACR measured with decay and sick leave as a true association.

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Table 1 (*Continued*)

Article:	Study design (from article)	Results	Authors' conclusions
Transmission either direct, via large aerosols (droplets) or small aerosols AND via fomite or hands:			
50	*Human controlled trials* Study of the transmission of the common cold under conditions similar to those of normal social interaction. Subjects: Volunteers in good general health, aged 19-45 y (141 females, 90 males). They were accommodated in pairs, or in single isolation for 3 d. Allocation to the experimental groups was randomized. Exposure / transmission: Five similar experiments were made, of the following 3 setups involving 82 volunteers and 22 donors. In 3 instances, children aged 8-13 y acted as donors, and in 2 others, adults were used. People with natural colds of recent onset were selected as donors. *Exposure to infected droplet nuclei:* A room of about 57 m^3 was divided in 2. A fan ensured good mixing of the air. Five or 6 healthy volunteers sat on one side of the partition; they were asked to read or to sew quietly throughout the experiment. Donors sat on the other side of the partition. The donors were encouraged to play games involving talking, shouting, or singing. Sneezing-powder was liberated halfway through the experiment. After a 2-h the donors left the room. *Full contact:* The same donors as in the first experiment mixed freely with a second party of healthy volunteers for a further period of 2 h, eating together and later playing cards and other games. *Indirect contact in contaminated environment:* The third group of volunteers was next exposed to the environment which had been contaminated by the donors in the full- contact exposure. Playing-cards and other objects handled by the previous occupants were again used for 2 h. Before the volunteers entered the contaminated environment, the room was aired for 10 min by opening windows and doors. Additional Exposure / transmission: *Transmission by means of mists:* the volunteer was instructed to inhale, artificial mists of infected particles through the nose and to exhale through the mouth (using a fitted mask). Exposure for 30 s, 3, or 5 min. *Contamination of exterior of nose:* Three different experiments were made in which infective secretions were applied only to the outside of the nose. Outcome: A clinical observer made a written assessment of the individual's symptoms. The nature of the exposure was not made known to the clinical observer	*Exposure to infected droplet nuclei:* 2 colds developed in 25 volunteers exposed to droplet infection. *Full contact:* Three in 32 exposed to full contact developed infection. *Indirect contact in contaminated environment:* Two in 25 exposed to a contaminated environment developed infection. The 2 colds in the last group were in volunteers occupying the same flat. One of these had minimal symptoms on arrival. Possibly a missed quarantine cold was responsible for both these infections. *Transmission by means of mists:* Seven of 32 exposed to infective mist developed a cold. *Contamination of exterior of nose:* No colds in 7 volunteers after virus was "painted" on the skin round the external nares. No colds developed as a result of 8 volunteers using contaminated handkerchiefs. No colds developed in the 7 volunteers who applied undiluted nasal washing over the outside of the nares (and repeated the process at short intervals for an hour).	Though common cold infection may spread from person to person by normal social contact, and through the air in the form of droplet nuclei, the rate of clinical cross-infection is low. We found no evidence suggesting that spread by indirect contact is of major importance in the natural transmission of the common cold. Our results suggest that the virus of common cold may be sensitive to drying. An analysis of the results has shown that an average of 50% of volunteers may be expected to develop colds after the instillation of infected drops into the nose, irrespective of age, frequency of natural colds, or time since last cold. From all the experiments together, we cannot conclude that children were more effective donors than adults. It appears that the placing of infective secretions round the outside of the nose is unlikely to produce a cold. The numbers do not permit a statistical comparison, but suggest that there has been inactivation of the virus as a result of the drying.
16	*Human controlled trials* Experimental induction of common cold via nasal drops or swapping. Subjects: Fifty-four human volunteers between (18 and 44 y old) at 2 different locations were inoculated via various routes with pooled nasal washings containing RV strain H.G.P. or Salisbury 1/57M. Exposure / transmission: Subjects were inoculated either as (1) nasal drops, or (2) swabbing (dipping a damp swab into undiluted virus and stroking it on the conjunctiva, on the anterior part of the nasal mucous membrane, or in the throat).	Colds were induced in 16 of 54 volunteers, after receiving virus as nasal drops, nasal swaps, conjunctival swaps or throat swaps. Twelve of 32 volunteers developed colds after given the virus by nasal drops, 2 of 7 volunteers developed colds after given the virus by nasal swabbing, and 2 of 7 after conjunctival swabbing. No colds were induced when the virus was given by means of throat swabs in 8 volunteers.	The final number of colds produced was not large; nevertheless it seems that colds may be produced almost as readily by applying virus by nasal and conjunctival swabs as by giving nasal drops, and also that the throat may be relatively resistant to infection.
51	*Human controlled trials* Effect of route of inoculation on experimental RV disease and evidence for airborne transmission. Subjects: Healthy adult male inmates from several correctional institutions and selected on the basis of serum antibody determinations and willingness to participate. Exposure / transmission: Volunteers were inoculated with RV NIH 1734 by small-particle aerosol or by nasal drops. Each man inhaled 10 liters through the nose, and exhaled by mouth into a discharge bag. Each inoculation required 30-60 s. The size of particles in the aerosol ranged from 0.2 to 3.0 μm in diameter. Nasopharyngeal inoculations were performed by the instillation of virus inoculum into each nostril of the volunteer (nasal drops). Outcome: Characterization of clinical response, dose-response, and influence of route of transmission.	For rhinovirus NIH 1734, afebrile upper respiratory tract illness occurred in volunteers regardless of inoculation method. Thirty-three of 41 (80%) were infected after aerosol exposure. Forty-three of 48 (90%) were infected after nasal drops. Lower respiratory tract illness was predominant in 5 of 41 volunteers who received small particle aerosol inoculation. All volunteers who received 0.1 $TCID_{50}$ by nasal drops became infected, although none became infected at 2 lower doses. The data suggest that the nasal mucosa is somewhat more susceptible to RV NIH 1734 than is the lower respiratory tract. Coughing and sneezing regularly produced quantities of virus sufficient to infect, whereas breathing did not.	It was concluded that both contact and airborne transmission of RV probably occur in natural circumstances, and that the predominant method of transmission may vary with the virus and with the particular environmental situation. Data on RV NIH 1734 indicates that either airborne or contact transmission would result in the upper respiratory tract illness characteristic of naturally occurring illness. Small-particle aerosol inoculation suggest that airborne transmission would produce a more varied response and account for the lower respiratory tract illness.

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Table 1 (*Continued*)

Article:	Study design (from article)	Results	Authors' conclusions
[52]	*Human controlled trials* Transmission of RV in a small Antarctic hut. Subjects: A wintering party of 17 British Antarctic Survey (BAS) personnel (men aged between 21 and 38). Exposure / transmission: After 5 mo of total isolation BAS personnel was inoculated under double blind conditions with placebo or RV type 2. 9 of the 17 men were given nasal drops containing RV type 2. 7 men were given a placebo. BAS subjects were not isolated from each other. Outcome: The clinical and virological responses of these subjects were compared with those of volunteers (healthy volunteers of both sexes aged 18-50) in England who received a similar dose of RV type 2.	Eight of the 9 men given RV developed symptoms within 48 h. Of the 8 close contacts given placebo or no inoculum, 5 developed symptoms from 5 to 16 d after the inoculation. Fifteen of the 17 BAS men shed virus on one or more occasions between inoculation and 17 days late. Men in Antarctic and the volunteers in England, given the same dose of RV2, were infected with similar frequency. Both the symptoms and the laboratory evidence of virus infection appeared to be more pronounced in the BAS subjects than in the volunteers in England.	An 88%-100% infection rate in 8 recipients occurred over 17 d among residents of a small hut in Antarctica. The men were crowded inside a small hut which almost certainly aided the spread of infection.
[53]	*Human controlled trials* Transmission of experimental RV in volunteer married couples. Subjects**:** Twenty-four childless couples (usually students) in which both members were seronegative to the challenge virus participated. Exposure / transmission: In the couples, 1 member was experimentally inoculated with a laboratory-grown RV virus strain (type 16 or type 55). Outcome: Quantification of virus in sequential (usually daily) samples from swabs of the anterior nares and of nasal washings, saliva, and hand washings, daily analysis of illness, and recording of the type and amount of contact were collected. The rates of intrafamily transmission was determined.	The rates of intrafamily transmission were 33% for the 12 couples in the experiments with R55 and 41% for the 12 couples in the experiments with R16. The overall rate was 38%. The presence of virus on the hands and anterior nares was significantly related to the capacity of a donor to transfer R16 to the spouse. Virus in saliva specimens was not so strongly linked to transmission. There appeared to be a definite relation between the number of hours spent in the same air space and transmission, but no relation was discerned between hours of direct contact and intrafamily virus spread. All couples in which transmission occurred spent at least 122 h together during a 7-d period.	Ability to transmit was directly related to time spent in the same air space. It seems likely that a considerable period must be spent together to create condition necessary for 1 person to infect another. There is the possibility that a combination of transmission routes are operative in the natural setting. Unless the infected partner had a moderate to severe cold and was shedding large amounts of virus the recipient spouse remained uninfected. In the great majority of donors, conditions for successful transmission occurred only on the second and third days after inoculation.
[54]	*Human controlled trials* Transmission of RV via hands or aerosols. Subjects: Healthy young adult volunteers (>17 y) with low or absent antibody titers to the challenge RV were recruited from the general University population. Exposure / transmission: Donors were inoculated intranasally with RV (strain HH). The same donors were used to expose volunteers in all recipient groups. Exposure took place on 3 successive days, the third through the fifth days after the first inoculation day of the donors. Three type of experiments were conducted between November and February. *Hand contact/self-inoculation exposure:* donors deliberately contaminated their hands with nasal secretions. Visible moisture or mucus was present on some but not all contaminated hands. Recipients then touched the hands of the donors for 10 s. Recipients were taken to another location where they inoculated themselves by deliberately placing their fingers on their nasal and conjunctival mucosa 2 or 3 times. This procedure was repeated on 3 successive days. *Large-particle aerosol exposure:* One donor and 2-4 recipients were seated around a table 70 cm in diameter in a room used only for this purpose. The donor was encouraged to talk loudly, sing, cough, and sneeze during the 15-min period of contact with recipients. *Small particle aerosol exposure:* Donors and recipients were housed in 1 large, closed room but separated by a double wire-mesh barrier that precluded any direct physical contact. Exposure was maintained continuously for 3 successive days and nights. Outcome: The rates of RV transmission was determined in the different settings.	Nine of the 11 artificially infected volunteers met the criteria of illness. Volunteers thought to be developing the most symptoms, especially rhinorrhea, on the day after the second challenge were selected as donors. Eleven of 15 hand-contact exposures initiated infection, compared with 1 of 12 large-particle and none of 10 small-particle exposures.	These experiments indicate that hand contact/self-inoculation is a very effective way to transmit experimental RV infection. In contrast, transmission of infection by large-particle aerosol was very inefficient in the study, and no transmissions were accomplished by small-particle aerosol.

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Table 1 (*Continued*)

Article:	Study design (from article)	Results	Authors' conclusions
[55]	*Human controlled trials* Transmission of RV from experimentally infected donors to susceptible recipients in a closed room. Subjects: Volunteers (>17 y old) were recruited from the University. In total 26 experimentally infected donors and 33 antibody-free recipients participated. Exposure / transmission: Donors were inoculated intranasally with RV. Three different exposure scenarios were examined: *Small-room experiments with RV55:* Five recipients were seated approximately 3 feet from either of 2 donors around a table in a small, unventilated room for 2 or 3 h. The volunteers played cards, sang, and conversed freely. The experiment was repeated 6 mo later with 4 recipients and 3 donors. *Dormitory-room experiment with RV55:* Eleven donors and 11 recipients were housed together for 3 consecutive days (12 h from 7 PM to 7 AM) in closed dormitory rooms. In order to decrease the likelihood of transmission by fomites, participants were asked to avoid handling one another's personal items and to use separate bathrooms. Five groups had 2 donors and 2 recipients, and 1 group had 1 donor and 1 recipient. All donors but 1 had mild-to-moderate colds, and all were shedding virus. *Kissing experiment:* Four donors kissed 5 recipients for 1 min 48 h after being infected with RV55. A second group of 6 donors, similarly infected, kissed 11 recipients for 1.5 min (two 45-s contacts). Each recipient was kissed only once; the donors and recipients were instructed to use the kissing technique most natural for them. Outcome: The rates of RV transmission was determined in the different settings.	*Small-room experiments:* No transmissions from 5 donors to 9 recipients occurred after 2-3 h of loud vocalization and card playing in a small room. *Dormitory-room experiment:* Three recipients developed colds, but only 1 case of transmission of RV was confirmed by laboratory findings. *Kissing experiment:* From 16 instances of direct oral contact between donors and recipients, only 1 instance of laboratory-confirmed transmission of RV occurred.	RV infections are difficult to transmit by short-term natural exposure, perhaps because the agent must be present in overwhelming numbers to reach susceptible mucosal cells. When transmissions do occur, the degree of donor symptomatology, the level of virus shed by the donor, and the amount of time that the donor and the recipient spend together appear to be important contributing factors. These results with lip and saliva specimens, along with the 8,000-fold lower level of sensitivity to infection via the tongue than via the intranasal route, may explain the difficulty of direct oral transmission of RV.
[56]	*Human controlled trials* To test the use of virucidal tissue to prevent dissemination of RV colds in a human volunteer model. Subjects: Volunteers comprised men (>17 y). Exposure / transmission: Twenty to 25 men were inoculated intranasally with RV16 on 2 successive days. On the third day, the 8 men with the most severe colds (donors) played poker with 12 antibody-free men (recipients) for 12 h. The experiments were carried out between 6 November and 16 June. *Virucidal tissues:* In 2 experiments, the donors and the recipients used virucidal tissues. Donors cleared their nasal passages gently into the tissues to avoid aerosol formation and to catch all effluent in the tissue folds. Any nasal excrement that might have escaped to the hands or to other surfaces was wiped with fresh tissues. *Control experiments:* In 2 control experiments, volunteers were permitted to behave naturally and to use cloth handkerchiefs for secretion, cough, or sneeze control as desired. All recipients changed location each hour to ensure equal exposure to each donor. The room was heated or air-conditioned by units that recirculated air within the room. Outcome: The rates of RV transmission was determined in the different settings. Colds were evaluated subjectively through a questionnaire filled out hourly by all participants.	None of the 24 recipients in the experiments with the virucidal tissues caught RV colds, whereas 5 (42%) and 75% (9) of the 24 recipients in the handkerchief groups became infected.	We have described the use of citric acid as a rhinovirucide when incorporated into a paper handkerchief and the complete interruption of transmission of R16 among participants in a 12-h poker game. In completely blocking transmission of R16 we did much better than expected, and we do not know what factors were required for this result. The rate of transmission appears to be chiefly dependent upon time spent together.

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Table 1 (*Continued*)

Article:	Study design (from article)	Results	Authors' conclusions
[33]	*Human controlled trials* Transmission of RV from experimentally infected donors to susceptible recipients in a closed room. Subjects: volunteers comprised men (>18 y). Exposure / transmission: Twenty-seven to 34 men were inoculated intranasally with RV16. Eight men with the most severe colds (donors) played poker with 12 antibody-free men (recipients) for 12 h. Experiments were done between 29 September and 9 November. Donor illness severity was maintained as donors were replaced from a pool of new RV16-infected men when their colds waned. Twenty-four adult men infected with RV16 (donors) and 36 susceptible adult male recipient played cards together for 12 h. Half of the recipients in each experiment wore restraining devices so they could not touch any part of the head or face. *Fomite transmission:* Twelve recipients played poker with cards, poker chips, pencils and other objects which had just been used (in a separate room) for 1-24 h by 8 poker-playing men with moderate to severe RV16 colds. Outcome: The rates of RV transmission was determined in the different settings. Colds were evaluated subjectively through a questionnaire filled out hourly by all participants.	There was no significant difference between the infection rate of the restrained and nonrestrained recipients. Overall, there was a 67% (12/18) attack rate in the control recipients, who could have been infected by all routes, and a 56% (10/18) attack rate in the restrained recipients, who could have been infected through the air (aerosols). *Fomite transmission:* None of the 12 recipients was infected by "grossly-handled" fomites (cards, poker chips, pencils and other objects).	At least in adults, it appears that RV colds are chiefly transmitted by aerosol and that natural transmission by fomites (indirect contact) is exceedingly difficult. These results point to aerosol transmission as the most important mechanism of natural spread of RV, at least among adults. Our present data suggest that natural transmission of RV by fomites, at least among adults, is quite unlikely.

nasal secretions were self-inoculated by a recipient.[37-39,54] However, a few studies questioned the relevance of RV transmission via fomites in natural settings, when it was demonstrated that significant amounts of fresh nasal secretions followed by self-inoculated were required.[33,36,47] As early as 1952 Lovelock et al wrote, reporting the results of one of the largest studies: "We found no evidence suggesting that spread by indirect contact is of major importance in the natural transmission of the common cold.".[50]

Another line of evidence for a specific route of transmission involves specifically interrupting transmission along the proposed route and showing that it reduces the incidence of infection. Using virucidal hand treatment or nose tissues Gwaltney et al showed that aqueous iodine (2%) applied to hands was effective in blocking transmission by hand contact of experimental infection with rhinovirus[37] and that virucidal tissues can reduce artificial transmission of RV.[39]

It can be questioned whether these experimental studies are relevant to how infection spreads in real-life; hence, Hendley et al conducted a comprehensive study over 4 years in which the mother in the family started virucidal treatment of her hands when a family member acquired a common cold. The results of this study supported the hypothesis that a proportion of natural common colds is spread by contaminated fingers.[40] The use of virucidal tissues, based on citric acid, was shown in an experimental model, to eliminate transmission of RV. However, no direct evidence for the dominant transmission route was shown.[56] A more recent study, found that use of a virucidal hand treatment, among young healthy adult volunteers, had no significant impact on the incidence of RV infection or RV-associated illness.[43]

We identified intervention studies where interruption of transmission route was evaluated in natural settings by increasing hygienic practices. Roberts et al documented a reduction in common colds in children under 24 months of age (but no reduction across the full age range) attending day-care centers following an intervention on hand hygiene.[41] In contrast 3 studies from Iceland, The Netherlands, and Denmark comprising interventions targeting increased cleaning of hands, toys, furniture et cetera, found no significant reduction in incidence rates of respiratory illnesses.[44-46]

So, to summarize the evidence for indirect transmission of RV by fomites and hands, we found moderate evidence for transfer in experimental settings, using large amounts of relatively fresh nasal secretions, but low evidence for this route in real-life. A single research group led most of the research more than 30 years ago and the question is to what degree the conditions under which the experiments have been carried out reflect realistic conditions in the general population. Bearing in mind the loss of RV infectivity on hands and surfaces[62] and the large dose of virus required to infect by self-inoculation of the nose or eyes, this route of transmission is probably only the dominant one in very special circumstances.

The mouth and throat may be relatively resistant to infection with RV. No colds were induced when the virus was given by using throat swabs in 8 volunteers[16] and indeed, kissing was found to be a surprisingly ineffective way of contaminating your partner. Only 1 successful transfer of RV during either 1 minute or 2 times 45 seconds of kissing between sick donors and 16 recipients.[55]

Airborne - studies addressing transmission, via large aerosols (droplets) or small aerosols

This route of transmission involves the generation of airborne particles (aerosols) containing infectious viruses. The particles can be large (often referred to as droplets; with a size of >10 μm) or smaller particles depending on the respiratory activities such as coughing, sneezing, speaking, singing, or just breezing, and the environment. Infection may be initiated when a new susceptible host inhales these aerosols and they are deposited in relevant sites in the respiratory system.

Table 2
Bias assessment of included articles

Article:	Study type	Selection bias*	Information bias†	Confounding bias‡	Evidence for mode of transmission:
Transmission by fomites and hands:					
[32]	*Laboratory experiments/ Human controlled trials*	*Moderate risk of bias* *Lack of control group* *Volunteers tend to be more health conscious than the general population.*	Serious risk of bias *Due to lack of molecular taxonomy identification of virus on hands, other surfaces and induced infections were not performed.* *No information of degree or passage of time of the disease.*	*Moderate risk of bias* *No information on possibility of other routes of transmission (airborne).* *Not verified that the same virus strain was the cause.*	*Some qualitative evidence for transfer and survival of RV on hands and surfaces.* *Small numbers of experiments.* *Significance in natural setting not clear.*
[36]	*Laboratory experiments / Human controlled trials*	*Moderate risk of bias* *No correlation between controls and cases was reported* *Volunteers tend to be more health conscious than the general population.*	*Moderate risk of bias* *No attempts were made to define the effect on virus survival of variations of temperature, humidity, or suspending medium.* *Mission information on RV type in different experiments.* *Mission information as the meaning of: "Inoculation and assessment of volunteers were double blind".*	*Moderate risk of bias* *No information on possibility of other routes of transmission (airborne).*	*Moderate evidence that viral contamination of the fingers is common in the acute stages of colds.* *Moderate evidence that infectious transfer via fomites are unlikely.* *Moderate evidence that infectious transfer via hands are unusual.* *Small numbers of experiments.* *Significance of these findings in relation to spread of colds under natural conditions is not known.*
[37]	*Human controlled trials - randomized*	*Moderate risk of bias* *Randomization process and group comparison not revealed.*	*Serious risk of bias* *Three of the recipients in the placebo-treated group acquired infection although virus was never demonstrated on their fingers. This result was probably because the cell cultures assay was not sensitive enough.* *Some possible observer bias as a result of the investigator's prior knowledge of the hypothesis under investigation.* *Small study – experiments on different time of year (November, January, March).*	*Low risk of bias*	*Moderate evidence that viral contamination of the fingers can transfer infectious rhinovirus as it can be interrupted with a viricidal agent.* *Significance of these findings in relation to spread of colds under natural conditions is not known.*
[38]	*Human controlled trials*	*Moderate risk of bias* *Volunteers tend to be more health conscious than the general population.*	*Serious risk of bias* *No detailed information on the amount and humidity of nasal secretion on surfaces (exposure).* *No detailed information on recipients' self-inoculation (realistic?).* *Infection was judged to have occurred if virus was recovered from a nose/throat culture and/or if there was a 4-fold or greater serum neutralizing antibody rise to the challenge virus.* *Some possible observer bias as a result of the investigator's prior knowledge of the hypothesis under investigation.*	*Moderate risk of bias* *No information about possible aerosol transfer.* *May not be a realistic scenario. Donors blew their noses into their hands and immediately held a coffee cup or rubbed a plastic tile with their fingertips for about 5 s.*	*Moderate evidence for rhinovirus transfer via nasal secretion.* *The infection rate of recipients contacting the tiles after disinfectant treatment, was not statistically significant different from non-treated tiles.* *May not be a realistic scenario. Donors blew their noses into their hands and immediately held a coffee cup or rubbed a plastic tile with their fingertips for about 5 s.*

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Table 2 (*Continued*)

Article:	Study type	Selection bias*	Information bias†	Confounding bias‡	Evidence for mode of transmission:
39	*Human controlled trials - randomized*	*Moderate risk of bias* *Randomization of donors not stated. Randomization process of recipients and group comparison not revealed.* *Volunteers tend to be more health conscious than the general population.* *Three recipients had to be excluded from analysis for various reasons.*	*Serious risk of bias* *No detailed information on the amount and humidity of nasal secretion on hands and recipients' exposure ("exposed to different individual donors on the consecutive study days").* *No detailed information on recipients' self-inoculation (realistic?).* *Some possible observer bias as a result of the investigator's prior knowledge of the hypothesis under investigation.* *Small study − experiments on 3 different time of year (November, January, March). No control group in November. Other differences between experimental studies.* *Greater efforts were made to ensure that the tissues were thoroughly wetted with nasal mucus. For example, placing some donors in the humid environment of the bathroom with the shower running, and by blowing the nose up to 3 times on the same tissue.*	*Moderate risk of bias* *No information about possible aerosol transfer.* *The conditions of this transmission model prevent extrapolation of these conclusions to the natural setting.*	*Moderate evidence for reducing transmission when using tissues; virucidal tissues in particular.* *The use of virucidal nasal tissues should reduce dissemination of virus in small and large particle aerosols from coughs and sneezes as well as hand contamination during nose blowing.* *Noses were blown with the same tissue up to 3 times to ensure wetting of the fingers with mucus that had passed through the tissue. May not be a realistic scenario.*
40	*Randomized human controlled trials*	*Moderate risk of bias* *The characteristic of the placebo solution differed from that of the active.* *Rate of compliance was 96% for the mothers using iodine and 52% for those using placebo (might have been an artifact due to more rapid fading on the skin of the placebo mixture).* *Volunteers tend to be more health conscious than the general population.*	*Low risk of bias* *Some possible observer bias as a result of the investigator's prior knowledge of the hypothesis under investigation.*	*Low risk of bias*	*Moderate evidence that interruption of transmission route by hands was achieved.* *Moderate evidence for indirect contact transmission by contaminated fomites and fingers.* *Relatively small number of evaluable episodes in each of the 4 study years and difficulty in blinding.*
41	*Human controlled trials − randomized cluster*	*Low risk of bias* *Day-care centers were comparable at baseline.*	*Moderate risk of bias* *Parents monitored the incidents of common cold in children.* *It is unclear whether any extra hand cleansing agents were used.* *It was not possible to blind the intervention.* *Parent reporting of illness may have been biased by knowledge of intervention trial.*	*Low risk of bias* *No information on possible airborne transmission.*	*Moderate evidence that improved hand hygiene can reduce acute upper respiratory infections in children under 24 mo who attend day-care.* *Indication of direct transmission of colds in young children in day-care.*
42	*Human controlled trials - double-blind, randomized*	*Moderate risk of bias* *Volunteers tend to be more health conscious than the general population. Subjects were compensated for participation.*	*Moderate risk of bias* *Some possible observer bias as a result of industrial funding and participation in study.*	*Low risk of bias* *No information about possible aerosol transfer.*	*Results confirm that infection can be induced by applying rhinovirus on fingers followed by self-inoculation via eye and nose.* *In trial 2, no significant reduction of infections was seen even though virucidal effect was documented.* *Minimal relevance for natural settings.*
43	*Human controlled trials - double-blind, randomized intervention*	*Moderate risk of bias* *Volunteers tend to be more health conscious than the general population. Subjects were compensated for participation.* *The clinical staff and subjects were not blinded to study treatment.* *Twenty-five subjects in the treatment group and 1 subject in the control group did not complete the study as planned.*	*Moderate risk of bias* *Some possible observer bias as a result of industrial funding and participation in study.*	*Low risk of bias*	*Moderate evidence that interruption of transmission route via hands did not mitigate number of RV disease in natural setting.* *Moderate evidence that the nature of the interpersonal interaction are important for the route of RV transmission.* *Transmission via hands not primary route in this population.*

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Table 2 (*Continued*)

Article:	Study type	Selection bias*	Information bias†	Confounding bias‡	Evidence for mode of transmission:
[44]	*Intervention cohort study*	*Moderate risk of bias* *The selection of DCCs for the intervention was based on their willingness to comply with the intervention protocol.* *The overall participation of children who delivered questionnaires during the study period was around 51%.*	*Moderate risk of bias* *A recall bias of the number of illnesses during the previous 6 mo is possible.* *It was not possible to blind the intervention.* *Parent reporting of illness may have been biased by knowledge of intervention trial.*	*Moderate risk of bias* *No information on other possible viral transmission route.* *No evaluation of incidence rates of illness compared to other DCC.*	*Moderate evidence that hygienic intervention does not reduce common cold when hygienic standards are high. Direct (contract) transmission not major transmission route in these settings.*
[45]	*Cluster randomized controlled intervention*	*Moderate risk of bias* *Thirty-nine percent of invited parents gave informed consent for their child to participate. 553 were eligible for inclusion and 545 participated. For 19 of 545 children, baseline incidence data were missing.* *Parents unwilling to participate may have less interest in hygiene which could have influenced disease incidence at child DCCs and possibly also the intervention effect.* *Half of intervention DCCs had already received the training on HH guidelines while baseline measurement was ongoing.*	*Moderate risk of bias* *Common colds comprised all infections agents giving rise to common cold symptoms.* *No identification of virus.* *Parents monitored the incidents of common cold in children.* *Study may be underpowered.*	*Low risk of bias* *Within DCCs other hygiene activities are important for the prevention of disease transmission.* *High hygienic standards may reduce the effect of intervention.*	*Indication that hand hygiene is not interrupting viral transmission.* *Common cold can be caused by several different virus; rhinovirus being the most common.* *Infection rate and hygiene practice at baseline may be important for effect of intervention.*
[46]	*Cluster randomized controlled intervention*	*Low risk of bias* *No information on randomization process.*	*Moderate risk of bias* *Parents monitored the incidents of common cold in children.* *Parent reporting of illness may have been biased by knowledge of intervention trial.* *The intervention was not blinded.*	*Moderate risk of bias* *Within DCCs other hygiene activities are important for the prevention of disease transmission.* *High hygienic standards may reduce the effect of intervention.*	*Moderate evidence that these fomites are not important in viral transmission in these settings.*
[47]	*Explorative observational study*	*Moderate risk of bias* *Recognizing disease can be challenging in nursing homes residents.* *No information on selection and allocation process.*	*Serious risk of bias* *No specific classification of common cold.* *The season for sampling virus correlated to disease not clear.* *Infections of residents were only followed over a 7-wk period.* *The differences in HH compliance levels between NHs were small, impacting the power of the analyses*	*Moderate risk of bias* *No data on viable virus on surfaces.* *No data and control for aerosol transmission.*	*Low evidence for lack of reduction of infections in nursing homes after hand hygiene intervention.* *No specific classification of common cold even though rhinovirus was 1 of 3 pathogen monitored.*
Transmission via large aerosols (droplets) or small aerosols:					
[17]	*Human controlled trials*	*Moderate risk of bias* *Volunteers from correctional institutions. No information on selection and allocation process (except that they were free of detectable neutralizing antibody).* *Inmates may not be representative for general population.*	*Low risk of bias* *The exposure was blinded for examining physicians.*	*Low risk of bias*	*High evidence that common cold can be induced via small aerosol exposure.* *Significance of these findings in relation to spread of colds under natural conditions is not known.*
[48]	*Human controlled trials*	*Moderate risk of bias* *Volunteers tend to be more health conscious than the general population.* *No information on selection and allocation process.*	*Serious risk of bias* *Symptoms relied on a self-administered questionnaire.* *Actual exposure dose of RV in eyes not clear.* *No control group.*	*Moderate risk of bias* *Dose may be too low*	*Moderate evidence that airborne transocular transmission of RV is not a common transmission route in short-term exposures.* *The potential differences between the tested human rhinovirus type 39 and other common cold viruses are unknown.* *Small study.*

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Table 2 (*Continued*)

Article:	Study type	Selection bias*	Information bias†	Confounding bias‡	Evidence for mode of transmission:
[49]	Observational study among randomly selected day-care centers	*Moderate risk of bias* *No intervention.*	*Serious risk of bias* *Child sick leave dates and underlying causes were registered by teachers* *The ventilation measurements were made 1 y after the registration of sick leave* No specific data on common cold or respiratory infections.	*Moderate risk of bias* *Increased ventilation may also reduce deposition of large respiratory aerosols on surfaces.*	*Moderate evidence of airborne transmission of disease as increased ventilation reduced sick leave.* *Increased ventilation may also reduce deposition of large respiratory aerosols on surfaces.* *All diseases are grouped together.*
Transmission either direct, via large aerosols (droplets) or small aerosols AND via fomite or hands:					
[50]	*Human controlled trials*	*Moderate risk of bias* *Volunteers tend to be more health conscious than the general population.*	*Moderate risk of bias* *Volunteers were isolated 2 or single for 3 d prior to exposure.* *Exposure consisted of natural (wild) colds with no identification of virus.* *No detailed information on ventilation and environmental parameters.*	*Low risk of bias*	*Moderate evidence for the finding that spread by indirect contact is of no major importance in the natural transmission of the common cold.* *Low evidence that the virus of common cold may be sensitive to drying.*
[16]	*Human controlled trials*	*Moderate risk of bias* *Two different volunteer-populations used.* *Volunteers tend to be more health conscious than the general population.*	Serious risk of bias *Exposure not precise.* *The virus used as an inoculum consisted of 3 pools of washings collected from volunteers at the onset of colds* *. . .the clinical observer, who did not know the nature of the inoculum, did not think that any of these " control " volunteers developed colds.* *Small size.*	*Low risk of bias*	*High evidence for induction of common cold via inoculation of nose and eyes.* *Significance in natural setting not clear.*
[51]	*Human controlled trials*	*Moderate risk of bias* *Volunteers tend to be more health conscious than the general population. Inmates may not be representative for general population.*	*Moderate risk of bias* *Volunteers were evaluated before inoculation and twice daily after inoculation by physicians who knew which volunteer was inoculated and which was an exposed susceptible.* *Volunteers were isolated 2 or 3 to a room for 3-4 d prior to inoculation and 10-14 d after inoculation.* *Only partial separation from the remaining camp population was maintained.*	*Low risk of bias*	*High evidence that artificial induced infections can initiate following aerosol exposure or direct nasal instillation.* *Moderate evidence suggest that the nasal mucosa is somewhat more susceptible to rhinovirus NIH 1734 than the lower respiratory tract.*
[52]	*Human controlled trials*	*Low risk of bias* *Antarctic personnel may not be representative for general population.*	*Moderate risk of bias* *Difference in virus inocula used in Antarctic and England.* *Inoculated under double blind conditions.* *Clinical effects were evaluated double blind.* *There were challenging experimental conditions in the Antarctic.* *Small study.* *No information on daily routines or assessment of possible RV transmission via contact.*	*Low risk of bias* *No data on transmission route.*	*High evidence for RV transmission among persons in prolonged close contact.* *No data on transmission route.* *Low evidence for airborne transmission.* *Significance of these findings in relation to spread of colds under natural conditions is not known.*
[53]	*Human controlled trials*	*Moderate risk of bias* *Volunteers tend to be more health conscious than the general population.* *No information on selection and allocation of donor (it seems like the husband appointed as donor).*	*Moderate risk of bias* *Symptoms relied on a self-administered questionnaire.* *Different types of RV used.*	*Low risk of bias*	*High evidence for transmission of RV between married couples.* *The design does not permit to differentiate fomite, hands or airborne transmission.*

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Table 2 (*Continued*)

Article:	Study type	Selection bias*	Information bias†	Confounding bias‡	Evidence for mode of transmission:
54	*Human controlled trials*	*Moderate risk of bias* *Volunteers tend to be more health conscious than the general population.* *No randomization, control or blinding.*	*Serious risk of bias* *Some possible observer bias as a result of the investigator's prior knowledge of the hypothesis under investigation.* *Only short term exposure in aerosol-experiments.* *Hand-contact and large-particle recipients were not placed in isolation.* *Large variation in donors shedding of RV.* *In 3 instances recipients became infected, although virus was never recovered from their hands.* *No virus was recovered from 2 of the 6 donors.*	*Moderate risk of bias* *No controls for possible aerosol-transmission in hand-contact experiments.* *According to Myatt et al*[61] *this study did not have sufficient power to detect airborne infection transmission.*	*High evidence for transfer of RV via hands in this model.* *The model may not be a realistic hand-to-hand transmission situation (repeated self-inoculation of fresh nasal secretions).* *Low evidence that aerosol transmission of RV is unlikely.*
55	*Human controlled trials*	*Moderate risk of bias* *Volunteers tend to be more health conscious than the general population.* *No information on selection and allocation process.*	*Moderate risk of bias* *Colds were evaluated by a questionnaire.* *No randomization or blinding.* *Large variation in viral shedding of the donors.*	*Low risk of bias*	*Small study – limited donor-recipient time together.* *Moderate evidence indicating aerosol transmission at low frequency.* *High evidence that infection via mouth is much lower than via the intranasal route.*
56	*Human controlled trials*	*Moderate risk of bias* *Volunteers tend to be more health conscious than the general population.* *No information on selection and allocation process.*	*Moderate risk of bias* *Some possible observer bias as a result of industrial funding and participation in study.* *Colds were evaluated subjectively through a questionnaire filled out hourly by all participants.* *No randomization or blinding.*	*Low risk of bias*	*High evidence that transmission of RV can be interrupted by virucidal tissue in this model.* *Transmission route not specified.* *The setting mimic a natural situation.*
33	*Human controlled trials*	*Moderate risk of bias* *Volunteers tend to be more health conscious than the general population.* *No information on selection and allocation process.*	*Moderate risk of bias* *Some possible observer bias as a result of the investigator's prior knowledge of the hypothesis under investigation.* *Colds were evaluated subjectively through a questionnaire filled out hourly by all participants.* *No randomization or blinding.*	*Low risk of bias*	*High evidence of RV transmission via aerosols in this model.* *Moderate evidence that RV transmission via hands and fomites not major route in this model.*

NOTE: Risk of bias for each domain is classified into 4 categories: low (study is comparable to a well performed randomized trial), moderate (study is sound for a nonrandomized study but cannot be considered comparable to a well performed randomized trial), serious (study has some important problems), and critical (study is too problematic to provide any useful evidence on the effects of intervention).

*Bias in selection of study population, randomization/selection, and the reported result.

†Bias arising because of misclassification of the level of exposure or factor being assessed and/or misclassification of the disease or other outcome itself. Risk of bias in selection of the reported result to be high if the study investigators had important conflicts of interest.

‡Bias due to a factor, which is independently associated with the exposure and the outcome.

We identified 10 studies addressing the possible airborne transmission. All studies display some bias from low to serious (Table 2). As early as 1952 it was demonstrated that 2 of 25 healthy volunteers seated on one side of a partition in a closed room were infected when donors with common colds were seated on the other side for 2 hours.[50] Further, it was demonstrated, that volunteers exposed to aerosols (0.2 μm-3 μm) containing RV became infected and displayed a disease similar to common cold.[17,51] Additionally, shedding of the virus in nasal secretions and to a lesser degree in oral secretions was documented.[63] An iconic study with British Antarctic Survey personnel who after 5 months of total isolation in Antarctica were inoculated under double-blind conditions with placebo or rhinovirus. The virus spread with a very high infection rate to those initially inoculated with placebo.[52] A smaller transmission rate was found in young married couples in which 1 member was experimentally inoculated with RV. In only 38% of the couples, the virus was spread to the spouse, and it was concluded that transmission required a significant amount of time spent together.[53]

Attempts to transfer RV via aerosols in different models have yielded conflicting results. D'Alessio et al were not able to demonstrate aerosol transfer in small-room experiments.[55] whereas Dick et al showed, that poker-playing volunteers transmitted RV at equally high frequencies whether their arms were restrained (no self-inoculation) or not.[33]

Further, indirect evidence for aerosol transmission involves the manipulation of the environment, for example, increased ventilation, air filtration, or social distance. A study from Denmark showed that ventilation, measured as air exchange rate, in day-care centers correlated to reduced sick leave among children. However, data on sick leave was not specific for RV.[49]

DISCUSSION AND CONCLUSION

That the common cold may be transmitted from person to person by the instillation into the nose of bacteria-free filtrates of infective nasal washings has been established as early as 1952.[50] The question is which route from the infected to the healthy nose the virus primarily is transmitted under everyday circumstances.

In the extensive research findings from the COVID-19 era, it has become clear that airborne transmission has contributed significantly more than previously thought for respiratory viruses.[7,64] In addition, we have learned that new mutations with new infectious properties continuously arise,[65] and this is particularly the case for RNA viruses such as corona-, influenza- and rhinovirus. This new knowledge gives us reason to reevaluate the characteristics of infection and possible prevention measures for the well-known viruses. Being the most common cause of upper respiratory tract infection, prevention of rhinovirus infections has enormous health and economic potential. The total cost associated with common cold in the United States was estimated to be approximately $40 billion per year (in 2003), which is at or above many significant chronic conditions including hypertension, chronic obstructive pulmonary disease, congestive heart failure, asthma, and migraine.[66] As development of a vaccine seems to have long prospects due to the many different antigenic variants, nonpharmaceutical interventions is our best measure for prevention. Knowledge of the most important routes of viral transmission in different situations, combined with research on costs and effects of interventions, allows prioritizing the prevention measures that provide the most cost-effective interventions.

There is no doubt that a common cold can be induced by applying rhinovirus directly in the nose of susceptible persons. Disease will develop in about 50% depending on the person's immune status, the dose, and strain of the virus. The question that has been discussed for more than 70 years is which transmission route is the dominant in real-life settings. Rhinovirus illness does not normally occur in epidemics but rather moves slowly through population groups probably because the infectivity between people is limited whether the location is the classroom, an industrial setting, health care facility,[67] or the family. Apparently, rhinovirus requires prolonged shared space in order to transmit efficiently via aerosols,[68] and this has recently been substantiated by the identification of low viral load in respiratory aerosols from individuals with symptomatic rhinovirus infection.[69] Interestingly, this study also found that for rhinovirus, unlike influenza and coronavirus, wearing a facemask does not protect from spreading the virus to the air.[69]

In the reviewed literature, we have found considerable disagreement regarding which transmission route is the dominant in real-life. The different results and theories might be influenced by the use of different strains of rhinovirus. Different viral strains may have different abilities for receptor binding, survival, multiplication, and viral shedding. Further, newer research has found significant differences in viral infectivity in different environmental conditions for example, the influence of temperature and relative humidity.[70] For example, RV has been found to be more infective at lower temperature in the nasal cavity, which is common in the colder seasons,[71] and only a few of the included studies specified the ambient temperature or relative humidity.

The disagreement regarding which transmission route is the dominant in real-life might also partly be explained by the virus capsid proteins which exhibit a high degree of heterogeneity, resulting in a wide antigenic diversity.[72] There are more than 160 known genotypes of RVs classified into A, B, and C species according to their phylogenetic sequence and distinct genomic features. RV-A and RV-C are more likely than RV-B to be associated with severe respiratory illnesses.[73] Each new infection contributes to the accumulation of immunity, which decreases symptom severity upon reinfection, and, returns from long school or university holidays is a frequent trigger for RV epidemic activity in the young adults. However, the large individual difference in immunity complicates the interpretation of many studies of virus transmission.

Synthesizing the results in the included studies shows a moderate evidence for transfer of RV via hands and fomites contaminated with large amounts of fresh, moist nasal secretions from a person actively shedding RV. The relevance of these studies in natural settings can be questioned, and we found low evidence for transmission via hands and fomites in natural settings. Some of the mentioned studies experiment with virucidal compounds and it should be noted that the toxicity of these might be significant. Information on the specific steps of viral transfer, which may play a role in rhinovirus transmission via hands and fomites, comes primarily from studies of volunteers infected by artificial means. Not all aspects of experimental infection may resemble those of naturally acquired infection. It cannot be excluded that this transmission route adds to the spread of RV in a special situation; for example, in day-care and private homes where close contact with sick children may be common. In a book chapter from 2001, the central researchers working on hand-transmission of rhinovirus during the 1970s and 1980s wrote "with rhinovirus, the best studied respiratory virus, an important proportion of infections (40%-60%) appears to result from the direct spread by hand contamination/self-inoculation.".[74] However, we consider repeated self-inoculation of fresh nasal secretions by adults uncommon.

We found moderate evidence that RV can be transmitted via aerosols in natural settings. This route requires significant doses of infectious virus particles and may require extended time in closed, poorly ventilated spaces. The efficiency of airborne RV virus transmission depends on viral shedding, factors that influence the aerosol concentration, such as ventilation, and those that affect the biological inactivation, such as the room air temperature and relative humidity.

During the COVID-19 era, it was remarkable, that the RV was the only seasonal respiratory virus to continue to propagate despite the use of facemasks, social distancing, and lockdown.[75-77] RV appeared to co-circulate with SARS-CoV-2 at a rate similar to its habitual seasonal patterns.[76,78] RV has been allocated to a seasonality profile termed *Perennial* (group 4), which circulate throughout the year, often with peak prevalence during autumn and spring.[79] We do not yet know whether the reason RV can flourish during the corona shutdowns is due to its ability to spread via aerosols, its seasonal type, its temporary, limited immunity, or the greater resistance to traditional disinfectants.

Knowledge of transmission routes is of utmost importance to mitigate the burden of respiratory viruses in daily life and to be better prepared for the next pandemic. This systematic review of the literature found low evidence for infectious transmission via hands in natural settings, but there is evidence that fresh nasal secretions can transmit RV infection in special situations.

The evidence for airborne (large or small aerosol) transmission in indoor settings is well documented. In some experimental settings, this transmission route was apparently the only one possible. The overall conclusion is that in natural everyday situations - in the office or in social gatherings - it is probably primarily the airborne transmission that is of greatest importance.

Implications

As the major transmission route of RVs in many indoor settings is through the air, preventive measures may focus on ventilation, occupation density, and other measures aiming at reducing aerosol generation and concentration.

Contamination of hands may also contribute to the transmission of RVs in some workplaces, institutions, and community settings. General hygiene and specifically hand hygiene are still central preventive measures for the reduction of viral transmission.

Several factors seem to influence the efficiency of RV transmission. First of all the nature of the specific RV strain, and the amount of virus particles in circulation The environment, with temperature, relative humidity, and possible UV-radiation has been demonstrated to have a large impact on the survival of RV and other viruses. Likewise, the air change through natural or mechanical ventilation and filtration will influence the concentration of potential infectious aerosols. Lastly, the immune status and possibly impaired respiratory protective function of the host contribute to the risk of infection.

Limitations

This systematic review has several limitations. Most of the studies included were published several years ago, and syntax, bias assessment, molecular techniques, and virus taxonomic were less developed. We may have missed some studies due to different naming and publication customs. We may be biased ourselves due to the overwhelming new knowledge on respiratory aerosols and airborne transmission which has emerged in the current corona era.

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.ajic.2022.12.005.

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