Routes of Transmission of the Influenza Virus 2011

Scientific Evidence Base Review

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Routes of Transmission of the Influenza Virus

Scientific Evidence Base Review

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This review was commissioned by the Department of Health in October 2010. The document was subsequently reviewed and endorsed by the Scientific Pandemic Influenza Advisory Committee (SPI).

In general, the review examined scientific literature published up until the end of 2010. This document thus represents a contemporary summary of the evidence base for the routes of transmission of influenza virus to humans, as of 2010. It is anticipated that additional informative studies in this area will be published over the course of 2011 and 2012. The review will therefore be updated periodically to reflect any additions to the scientific literature that might alter any of its conclusions.

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Executive summary

1. There is sound evidence supporting influenza virus survival on fomites and hands for periods consistent with the possibility of onward transmission.
2. The data are relatively heterogeneous regarding the likely survival time of virus deposited on surfaces and factors such as virus concentration of the inoculum, type of surface and temperature and humidity clearly affect virus survival. Thus, it is not possible to provide absolute numbers or ranges for survival times further than to say that estimates lie in the range of a few hours to several days.
3. In general the data support longer survival on hard (non-porous) surfaces than on softer (porous) items.
4. Few data demonstrate the recovery of viable virus from surfaces contaminated by patients with natural or experimental influenza compared with recovery of viable virus from surfaces after deliberate inoculation. This might reflect limitations in sampling efficiency, study designs, or virological techniques and does not reliably indicate that contact transmission is relatively less important than droplets/aersosols.
5. The indirect contact route of transmission is the most vulnerable to natural interruption because it involves multiple stages. In order for infection to be transmitted; a) titres of virus in excess of the human infectious dose must be shed, b) deposited virus must survive, c) high titres must be collected via hands, d) virus must survive on hands, e) hands must deposit an infectious dose of virus on target cells.
6. Whilst there are unanswered questions about the relative importance of contact transmission compared with other routes, contact transmission cannot be excluded.
7. Coughing and sneezing produce a ‘respiratory spray’ consisting of large particles (droplets) and small particles (aerosols).
8. From the available evidence there is no doubt that droplet transmission of influenza occurs.
9. Aerobiological studies reveal that the vast majority of pathogens excreted during human coughing and sneezing are contained within droplets.
10. These particles behave ballistically and fall out of circulation within a few feet (range is proportional to size).

11. This does not necessarily imply that droplet transmission produces the greatest number of secondary infections; in order to be ‘effective’, droplet sized particles in coughs and sneezes must be targeted towards fomites or towards a susceptible contact.
12. Droplet particles will not penetrate as deeply into the pulmonary tree as would aerosol particles and other data suggest that deeper lung deposition of influenza virus may be more potent in initiating infection, and a lower inoculum may be needed.
13. Although the majority of particles produced lie in the size range that most authorities would regard as aerosol-sized, somewhat paradoxically, only a minority proportion of the total pathogens excreted will be contained within aerosol-sized particles (perhaps as few as 1%); a reflection of their relative volume.
14. By inference, the likelihood of infectious aerosol particles being produced is probably increased in patients who are shedding higher virus titres (those in the early days of the illness, children, immunocompromised patients, those with a frequent cough/sneeze)
15. Thus, the degree of heterogeneity regarding the production of infectious aerosols might be considerable in human subjects infected with influenza.
16. With regard to aerosol transmission, there is evidence from challenge studies that a lower infectious dose of influenza might be needed if virus deposition occurs deep in the pulmonary tree via aerosol particles, than inoculation via nasal drops; the resulting illness from aerosol inoculation also seems more severe.
17. There is good evidence for aerosol transmission of influenza from animal models; the extent to which these findings can be generalised to human transmission is uncertain and scientifically challengeable.
18. Although there is an absence of good quality epidemiological data to support long-range transmission of influenza via aerosols (suggesting that this phenomenon is rare or non-existent) these data need to be placed in the context of the rapid diminution of concentrations of infectious aerosols as distance from the generating source increases.
19. Thus, the absence of evidence for long-range transmission does not preclude a significant role for short-range spread via aerosol-sized particles, in some circumstances, at ranges normally or traditionally attributed to only ballistic-sized larger droplets.
20. Outbreak studies are inconclusive in determining the relative importance of different modes of influenza transmission. They suggest that most influenza transmission occurs at close range but multiple modes of transmission are possible including contact, droplet and via aerosols.

21. Studies of the comparative effectiveness of surgical face masks and respirators are inconclusive to date and cannot be extrapolated to draw conclusions about modes of transmission. For example, surgical face masks may act as a droplet barrier and a 'no-touch-face' device – i.e. they might interrupt both droplet and contact transmission.
22. The evidence for hand hygiene is considered in a separate paper.
23. At present, the existing evidence on influenza transmission supports a potential role for contact, droplet and aerosol transmission.
24. The evidence base is insufficiently clear to determine the relative contribution of contact, droplet and aerosol transmission and justifies the continued emphasis on respiratory and hand hygiene in public education materials.
25. However a role for aerosol transmission from some infected individuals in the absence of known aerosol generating procedures cannot be ruled out and a lack of evidence of long-range influenza transmission is not adequate evidence of absence of aerosol transmission at shorter distances.
26. In healthcare settings the use of high-level respiratory protection (FFP3* respirators) for known aerosol generating procedures performed on patients infected with influenza remains appropriate.
27. Use of protective equipment for other close range healthcare contacts with influenza patients also remains appropriate.
28. In the absence of performing procedures that are known specifically to be aerosol generating (e.g. during routine close range patient care), aerosol transmission might still occur; in these circumstances surgical face masks would not be fully protective.
29. The evidence is insufficiently clear to identify the relative contribution of aerosol transmission in the absence of performing procedures that are known specifically to be aerosol generating, and insufficiently clear to identify a subset of patients from whom aerosol transmission is more likely. Though, by inference, this is most likely to be in people prone to high virus shedding (children, immunoncompromised people) and early on after symptom onset, but direct evidence is lacking.

*Scientifically FFP2 standard (US equiv N95) respirators are likely to be adequate for the prevention of aerosol transmission of influenza but the UK regulatory framework set by HSE only permits the use of FFP3 standard equipment (US equiv N99).

Glossary

Aerosol	A gaseous suspension of fine solid or liquid particles. An aerosol can consist of a range of particle sizes; small particles will remain suspended in the air for prolonged periods of time (droplet nuclei) and larger particles (droplets) will quickly settle to the ground. In this review the term aerosol transmission will refer to the transmission of infection mediated by droplet nuclei only
Airborne	Carried by or through the air
Bioaerosol	A gaseous suspension of fine solid or liquid particles that are living, contain living organisms or were released from living organisms
Contact	The transfer of an infectious agent from one being to another by a coming together or touch. *Direct Contact*: transmission via direct physical contact; for example a kiss. *Indirect Contact*: transmission via an intermediate object such as a fomite
Droplet	A particle >10μm and <500μm
Droplet nuclei	A particle ≤10μm
Face mask	A protective covering for the mouth and nose Whilst it will provide a physical barrier to large projected droplets, it does not provide full respiratory protection against smaller suspended droplets and aerosols
Fomite	An inanimate object or substance capable of carrying infectious organisms
Inhalable	Particles that enter the body through the nose and/or mouth during breathing. They do not travel further than the tracheobronchial tree
Respirable	Inhaled particles that penetrate to the alveolar region of the lung
Respirator	A protective covering for the mouth and nose. It provides a high level of filtering capability and face fit. *FFP2 / N95*: respirators that are able to filter out particles of >0.3μm with an efficiency of 95%. *FFP3 / N99*: respirators that are able to filter out particles of >0.3μm with an efficiency of 99% FFP is a European classification system whereas as N is the US ‘broad equivalent’; testing protocols are not identical and the ratings are not directly interchangeable

Abbreviations

AR	Attack Rate
ARI	Acute Respiratory Infection
CDC	Centre for Disease Control
ECDC	European Centre for Disease Control
FFP	Filtering Face Piece
HCW	Health Care Worker
HH	Hand Hygiene
ID_{50}	Infectious Dose (that causes infection in 50%)
ILI	Influenza Like Illness
LRT	Lower Respiratory Tract
NI	Neuraminidase Inhibitors
NPI	Non Pharmaceutical Interventions
PCR	Polymerase Chain Reaction
RH	Relative Humidity
RSV	Respiratory Syncitial Virus
SAR	Secondary Attack Rate
SFM	Surgical Face Mask
SRAT	Short Range Aerosol Transmission
$TCID_{50}$	Tissue Culture Infectious Dose (that causes infection in 50%)
URT	Upper Respiratory Tract
URTI	Upper Respiratory Tract Infection
UV	Ultra Violet
WHO	World Health Organization

Introduction

Despite the fact that influenza has impacted on human health for at least several centuries *(1)* and that the virus was first identified in humans in 1933 *(2)*, remarkably little is known definitively about its modes of transmission. Thus, important health policy and infection control issues remain unresolved; for example, how effective surgical masks or respirators might be reducing transmission. These shortcomings have been exposed in national and international pandemic preparedness activities over recent years and during the 2009 H1N1 pandemic itself. Indeed, the ECDC, WHO, and the U.S. Institute of Medicine have prioritised understanding the modes of influenza transmission as a critical need for pandemic planning *(3-5)*.

A sound understanding of the basic science of influenza transmission is key to developing evidence-based policies for infection prevention and control. At present opinions are sharply divided on the importance of aerosol versus droplet transmission *(6-8)*. The uncertainty about the importance of different mechanisms of influenza transmission and the best means to prevent spread is no more clearly reflected than in the diverse approaches adopted by different countries in relation to the use of face masks by healthcare workers and the public, and adoption of different 'safety distances' for the radius of potential spread from an infected person. For example, the UK (in line with WHO guidance *(9, 10)*) recommends droplet as opposed to aerosol infection control precautions (primarily surgical face masks (SFMs) rather than respirators) for healthcare workers for most close contact (within one metre) with pandemic influenza patients *(11)*, whereas US *(12, 13)* and French *(14)* guidance recommends respirators for all forms of close contact (within six feet). At present there is little in the way of firm evidence with which to formulate guidance for healthcare workers as to the level of risk reduction provided by different types of protective equipment.

The evidence base on influenza transmission is largely derived from six core categories of study:

1. Studies assessing influenza virus deposition and survival in the environment
2. Studies examining the epidemiology of disease in hospitals, nursing homes and other closed or semi-closed settings. From these data, inferences are drawn about modes of transmission that could have produced the pattern of disease observed

3. Prospective pharmaceutical and non-pharmaceutical intervention (NPI) studies in the setting of natural infection
4. Animal models of transmission; information generated from experimental studies in different animal models can provide useful insights, however, any extrapolation to humans relies on assuming transmission mechanisms and behaviours are similar in humans and other animals
5. Human influenza challenge studies; infection, initiated by a number of routes, and subsequent patterns of virus shedding have been described for experimentally infected individuals in a relatively small number of studies
6. Modelling has been used to explore the relative contributions that each route of transmission may have

This paper is not a formal systematic review of influenza transmission. It is a review of all lines of evidence (mentioned above) that contribute to an understanding of the different routes of transmission that operate in humans. This includes examining whether proposed routes are biologically and scientifically plausible, considering factors which may influence this and appraising a body of literature concerning influenza infection (in humans and animals) from which evidence about modes of transmission can be drawn. It concludes by assessing the relative importance of the different routes with the aim of informing pandemic preparedness policies for infection control and prevention and advice to citizens.

Definitions

One of the difficulties that arises in the reviewing the literature on influenza is the inconsistency and variety of terms that are used to refer to the modes of transmission. Traditionally the standard definitions used by the Centers for Disease Control (CDC) to describe modes of transmission (not specific to influenza) have included *(15)*;

- Direct Contact – transmission via direct physical contact; for example a handshake or kiss
- Indirect Contact – transmission via an intermediate object such as a fomite
- Droplet – droplets are particles >5μm and are generated from the respiratory tract. They act like ballistic particles and hence some view them as a form of direct contact
- Airborne – transmission by bioaerosols; particles <5μm that can remain suspended in air, travel long distances (>6ft) and can deposit in the lung.

Airborne transmission has generally been used to refer to infections that spread over long distances through particles in the air, for example tuberculosis. Only droplet nuclei in bioaerosols (aerosols that contain living organisms) remain suspended in the air and can travel over long distances but some confusion can arise because; i) droplets fall within the definition of an aerosol and could be considered to be airborne (although only for a short period of time and over short distances) and ii) droplet nuclei can transmit infection over short distances as well as long; in fact, because droplet nuclei are more concentrated nearer their source, they are more likely to transmit over short distances than long. In addition, it should be recognised that there is no absolute cut-off between droplet nuclei and droplets; particles lie on a continuum, with larger particles tending towards droplet behaviour.

Another level of complexity is introduced by considering the aerobiology of particles in aerosols, in particular their penetration of the respiratory tract. For example particles >10μm can be inhaled but not respired, i.e. will not penetrate into the alveolar region of the lung *(16)*. Furthermore, all aerosolised particles are dynamic, that is they change size as water is exchanged (taken up or released) with the atmosphere; this is dependent upon factors such as humidity, temperature and airflows.

The following terms (which are based on working definitions used by Weber and Stilianakis in a review of influenza transmission *(17)* and those used at a recent CDC workshop on influenza transmission) will be used in this review (Figure 1).

- Droplet transmission: Transmission of influenza through the air by droplet particles (>10μm) emitted by an infected host (e.g. by coughing) which deposit on mucous membranes either directly or by inhalation. It is likely that an infectious virus particle will reach its target cell by inhalation more commonly than by direct contact.
- Bioaerosol transmission: Transmission of influenza through the air by droplet nuclei (≤10μm) which can be respired. Particles penetrate distally into the lung and initiate infection there.
- Contact transmission:
  - Direct Contact – transmission via direct physical contact; for example a kiss
  - Indirect Contact – transmission via an intermediate object such as a fomite

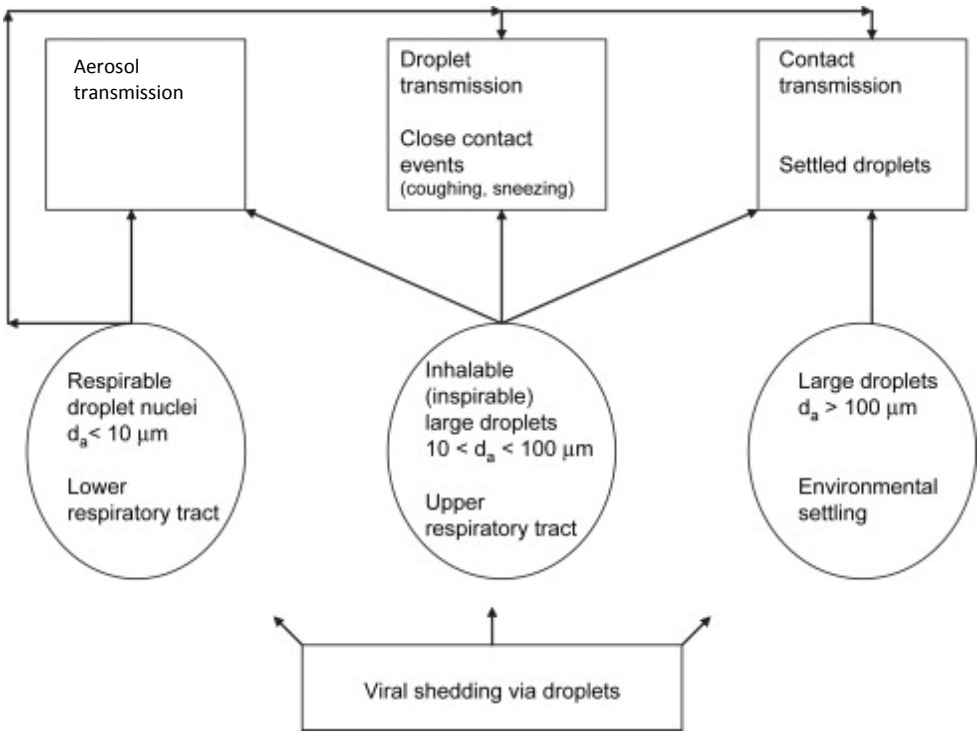

Figure 1: Adapted from Journal of Infection with permission from Elsevier: Weber and Stilianakis. Inactivation of influenza A viruses in the environment and modes of transmission 2008, (57); p361-373. Classification of respiratory droplets and modes of influenza transmission. Both inhalable and respirable particles can contribute to all three transmission modes. Large droplets with an aerodynamic diameter above 100μm are not inhalable, will settle on surfaces within a few seconds of being expelled and can thus only contribute to contact transmission.

Search Methods

Dr Ben Killingley conducted this review. He is an MRC clinical research fellow and infectious diseases physician currently undertaking a PhD on influenza transmission at the University of Nottingham. Contributions have been made by Professor Jonathan Van-Tam (University of Nottingham and HPA -document oversight and executive summary) and Allan Bennett (HPA - aerobiology of influenza).

For most chapters, a PubMed search using specific search terms has been performed to help identify all relevant evidence. Articles were selected by reviewing titles, appraising abstracts and reading the full text of papers. Only studies published in English and which had an abstract were included. Further articles were identified by extended searches on PubMed that related to chosen articles and the author's personal reference collection, including a review of article bibliographies.

Data synthesis has taken the form of a narrative approach that includes an appraisal of the evidence presented including the strengths and weaknesses of specific studies with regard to their ability to contribute to the knowledge base on routes of influenza transmission. In addition, the synthesis considers the implications for policy as well as future research.

1. Studies assessing influenza virus deposition and survival in the environment

It is important to establish at the outset whether the proposed routes of transmission are scientifically plausible. This chapter will consider what is known about the aerobiology concerning virus emission from humans, and subsequent virus deposition and survival in the environment.

Influenza replicates in epithelial cells throughout the respiratory tree (both upper and lower tracts) *(18)*. Human viruses preferentially bind to cell surface receptors terminating in an α(2,6)-linkage in contrast to avian viruses which prefer an α(2,3)-linkage *(19)*. The predominance of these receptors in different tissues reflects the tropism seen, e.g. α(2,6) are found mainly in the human respiratory tract *(20)*. As a result both virus entry and exit in humans occurs through the respiratory tract i.e. mouth and nose. Virus emission occurs via mechanisms such as coughing and sneezing which produce a 'respiratory spray' of different sized particles on which virus travels. In this review large particles (>10μm) are classed as droplets and small particles (≤10μm) as droplet nuclei. Virus entry occurs by respiration (droplet nuclei) and/or inhalation (droplets) and/or direct contact (droplets) or indirect contact (settled droplets and droplet nuclei). The potential of the conjunctiva to mediate transmission of human influenza viruses remains uncertain *(17)* though data from tropism experiments with pandemic H1N1 *(21)* and outbreaks of avian H7 viruses in humans that are marked by conjunctivitis confirms the presence of α(2,3) receptors in the eye *(22)*. There is very little evidence to suggest that the faecal-oral or waterborne route of transmission occurs in humans which contrasts with transmission in birds *(23, 24)*.

Search Methods

Contact transmission - A PubMed search was undertaken on 22/10/10. The search terms 'influenza' AND 'transmission' AND 'fomites', 'environment' and 'hand' generated 488 citations. Eleven titles were found to be appropriate for further review and the abstracts were read. Eight

full articles were read and selected for discussion. Expanded searches and personal collections generated three further articles that are also considered.

Droplet and aerosol transmission - The authors (BK and AB) own reference collections, relevant review papers and bibliographic searches of selected articles were used to identify studies. In addition, PubMed searches looking for related citations to those already selected were performed.

Contact transmission

For contact transmission to occur; i) viable virus is released from a host; ii) virus must survive for a period of time on hands +/- fomites; iii) an infectious dose must be delivered to a site where infection initiation can occur. The evidence that virus can survive (i.e. remain viable and infectious) in the environment and be transmitted via indirect contact is reviewed below. N.B. viable virus can be detected by culture and PCR methods, however PCR can also detect non-viable virus. It is not possible to determine whether a virus is viable or not on the basis of a positive PCR result.

Virus survival – Hands

- In a study by Grayson et al, a relatively high dose of an H1N1 virus (10^7 $TCID_{50}$/0.1ml) was used to contaminate the hands of 20 volunteers. After two minutes, a reduction in virus as measured by PCR was seen and virus was cultured from the fingertips of 14 volunteers (a 3-4 log reduction in virus $TCID_{50}$ was seen). Eight volunteers were assessed after 60 minutes; little further reduction in virus levels (assessed by both culture and PCR) was seen. Various hand hygiene methods were used to cleanse hands after two minutes; no virus could be cultured and all lead to significant reductions in PCR copy numbers *(25)*

- Thomas et al performed a study that involved contaminating the fingertips of six volunteers with 2mls of either an H3N2 or an H1N1 virus mixed with respiratory mucous (5.8×10^7 and 2.5×10^6 $TCID_{50}$/ml respectively) *(26)*. Virus detection was undertaken by culture. In the first part of the study the effect of time was assessed; after one minute virus was detected on all (36) fingertips contaminated with either virus, after five minutes 5/18 and 8/18 and

after 30 minutes 2/18 and 2/18 fingertips were positive for H3N2 and H1N1 respectively. It was then shown that bigger volumes of inoculum led to more virus being detected at 15 minutes (2mls = 2/18, 5mls = 6/12 and 30mls = 9/12). Finally it was shown that if the viral inoculum was spread on the fingertip (rather than being left as a drop) survival was lessened; H3N2 - one minute 12/18 v 18/18, five minutes 0/18 v 10/18; H1N1 – five minutes 3/18 v 8/18

- Bean et al showed that virus can be transferred from deliberately contaminated fomites (stainless steel surface and tissue) to hands. After an initial inoculum of $10^{5.6}$ $TCID_{50}$/0.1ml, $10^{4.5}$ and $10^{3.0}$ could be transferred immediately from the steel surface and tissue respectively. Within five minutes titres of virus on hands had fallen to $10^{1.3}$ and $10^{1.0}$. Virus could barely be detected on hands after it had been present on the tissue for 15 minutes whilst it could be detected after being present on the steel surface for >24 hours *(27)*

Virus Survival – surfaces

- As part of the study above, Bean et al demonstrated that influenza A (H1N1) can survive for prolonged periods (48-72 hours) on hard non-porous surfaces, for example stainless steel and plastic, whereas it survives for shorter periods of time (8-12 hours) on porous surfaces such as tissues, handkerchiefs and magazines where inocula drying times are shorter (10 vs. 90 minutes) *(27)*

- The survival of viruses (H3N2 and H1N1) has been assessed on banknotes. It was shown that the recovery rate was directly related to inoculum size; 8.9 x 10^5 $TCID_{50}$/ml could be isolated (by culture) at two days compared to 1.1 x 10^5 $TCID_{50}$/ml which could only be isolated for up to one hour. It was then revealed that the addition of respiratory mucus to inoculums increased the duration of infectiousness, e.g. eight days vs. two hours for an H3N2 virus. The authors then went on to show that virus contained in nasopharyngeal secretions obtained from ill children survived on banknotes for at least 24 hours in 50% (7/14) and at least 48 hours in 36% (5/14) *(28)*

- Virus survival on a range of representative household surfaces has been studied. Surfaces such as stainless steel, polyvinyl chloride (light switch), wood, glass, computer keyboard, soft toy and J cloth were used. 9 x 10^9 pfu/ml of a laboratory H1N1 virus was inoculated onto these surfaces and then sampled at set time points using a cotton swab. Viable virus

could be recovered from most surfaces four hours after inoculation although differences between porous (less survival) and non porous surfaces were evident. However, viable virus could not be detected on any surface other than the plastic (Petri dish) control nine hours after inoculation. Similar results were found when a 2009 pandemic H1N1 strain was tested although it survived for longer (9-24 hours) on glass and a kitchen work top (personal communication Dr J Greatorex)

- Influenza virus survival is affected by temperature, relative humidity (RH) and exposure time after being deposited on a stainless steel surface *(29)*. Drying in ambient conditions (temperature 24^0C, relative humidity 35%) for an hour resulted in a reduction of 63%. It was shown that viral inactivation increased with rising temperature (55 → 60 → 65^0C) and RH (25 → 50 → 75%). The data suggested that absolute humidity was a better predictor of virus inactivation than RH, in keeping with a previous observation *(30)*

Detecting virus in natural conditions

Hands

As part of randomised trial in Thailand to investigate hand hygiene and surface contamination during the 2009 pandemic, the hands of index cases (infected children) and secondary cases within the household were swabbed (on day three). Forty-five households were recruited to a hand washing arm (they received HH education and liquid soap) and 45 to a control arm (no specific HH instruction). The hands of 15/90 (16.6%) index cases were positive by PCR, one (1.1%) was culture positive, whilst 1/59 (1.6%) secondary cases were PCR positive and none were culture positive. Amongst the index cases there were no differences in positivity rate between the two arms (7/15 HH vs. 8/15 control) *(31)*.

Fomites

A number of studies have attempted to assess virus contamination of fomites in the near environment of infected (or potentially infected) patients.

- A number of fomites in households were swabbed in the study conducted by Simmerman et al mentioned above; 540 swabs were collected, 3% were positive by PCR. 16/90 (17.8%) households had at least one fomite positive by PCR; 11 control, five HH (prevalence risk difference = 13.3%; 95% CI -2.2 – 28.9%; P=0.09). No swabs were culture positive. Households in which the index case was <8 years old had a significantly higher prevalence of contamination *(31)*.

- Boone and Gerba *(32)* collected 218 swabs from fomites in 14 child day care centres and 92 swabs from the homes of eight children (five of the homes had at least one child with flu-like symptoms). The authors noted a seasonal variation; swabs taken in spring (coinciding with the flu season) generated higher positivity rates. Overall, influenza was detected (by PCR) from over 50% of all swabs taken during the influenza season (53% in day care centres, 59% in homes). Virus culture was not attempted.
- Boone and Gerba were also involved in a study that assessed the presence of influenza virus (by PCR) on fomites in school classrooms during a flu season. Fifty-four swabs were taken from three classrooms, 13 (24%) were positive; it was detected most commonly on student desktops *(33)*.

- Fomites were swabbed during a study that involved subjects who were experimentally infected with an H3N2 influenza virus. Samples taken from fomites in subjects' rooms revealed influenza (detected by PCR) on 9/48 swabs (19%), though no live virus was found [personal communication Dr B Killingley].

- 397 fomite swabs were collected from the homes and hospital rooms of confirmed influenza patients as part of a study during the 2009 pandemic. Virus was detected by PCR on two occasions, on two surfaces from one patient in their own home (following discharge from hospital), giving a swab positivity rate of 0.5%. Live virus was recovered from one surface (kettle handle). The subject from around whom the swabs were taken was shedding live virus from the nose on the same day, though other household members were also symptomatic *(34)*. Hand hygiene and its effect of transmission is discussed in chapter 3

Discussion

There exists significant heterogeneity in the design and methods of the studies discussed and it is difficult to draw unifying conclusions. Variations take the form of; virus strains examined, concentrations of inocula used, manner of inoculation, populations studied, environmental conditions, sampling methods and detection techniques. Efficient sampling and detection are vital as viable virus is easily lost during experimental manipulations; only the study by Bean et al gives estimates of their sampling efficiencies (83 – 97%). Such efficiencies are likely to be considerably less outside the setting of controlled laboratory experiments. Furthermore, whilst

laboratory based studies are useful for defining parameters of what may be possible, the relationship between these studies and what happens in 'natural' conditions is difficult to judge. Thomas et al attempt such a comparison in their study; it appears that virus from patients survives less well (50% do not survive >24 hours) compared to the lowest dose of experimental virus inoculated with mucus (>50% survive 5 days). At the patient level, inter and intra variation complicates the issue; patients will shed virus at different titres during the course of their illness, some will patients will shed more than others and environmental conditions (e.g. temperature and humidity) may differ.

Despite some limitations, there is good evidence to confirm the ability of influenza A to remain viable on fomites. Survival on hard non-porous surfaces where drying times are longer than those of porous surfaces usually extends well beyond 24 hours. However, the ability to survive does not necessarily equate to the ability to infect. In the three studies that sought out live virus, only one single swab was positive. This suggests that either swabbing and detection methods are insensitive or that virus deposited by infected patients does not contaminate the vast majority of fomites in high titre.

Similarly there is evidence that virus can survive on hands for at least five minutes. Although survival on hands appears significantly reduced compared to some fomites this may not be significant if hands frequently 'collect' virus and then deposit virus on a mucous membrane (face touching has been shown to occur at a rate of 15.7 events per hour *(35)*).Yet again however, when we analyse (the albeit limited) findings from the field, the potential for transmission via hands is not supported; only one out of 90 cases had viable virus detected. Another obstacle is that the infectious dose of influenza transmitted in this way is not known. Even if viable virus is detected, is enough of it present to cause infection?

In spite of these reservations, based on their data and making certain assumptions (e.g. a 50% human infectious dose = 30-127 $TCID_{50}$ and the transference of a 0.01-0.02ml inoculum from surface to hand) Bean et al conclude that a person shedding large quantities of virus (>$10^{5.0}$ $TCID_{50}$/ml) could transmit infection via stainless steel for two hours and via tissues for a few minutes *(27)*.

The contact route of transmission cannot be excluded; virus survival data shows that it is plausible. Its importance however, is questioned by field data (although the scarcity and

uncertain quality of the field data itself is problematic). More data from infected patients in real-life circumstances are needed.

Droplet Transmission

This route of transmission is reliant on close contact so that a droplet carrying infectious virus, expelled from an infected individual, comes into contact with the respiratory tract of a susceptible individual. It is mediated by large droplets (normally considered to be ≥10μm and detected up to size of 500μm) which behave like ballistic particles after being generated by activities such as coughing and sneezing *(17, 36, 37)*. It has been shown that over 99% of pathogens emitted in a cough are carried by particles >100μm *(38)*. The distance these particles travel is determined by their initial velocity, their terminal velocity and gravitational acceleration; it has been assumed that particles >150μm can travel >60cm *(36)*. So, although the majority of droplets expelled during a cough or sneeze will settle to the ground quickly and not reach a susceptible host, they remain potentially important as the few droplets that do reach target cells can carry a high pathogen load. They reach respiratory epithelial cells via direct contact or inhalation; the latter is perhaps more likely to deliver an infectious particle than contact as the probability that a cough or sneeze is perfectly directed so that particles land directly on them is small *(36)*. However, the inhalation of particles following a cough or sneeze is dependent on several factors such as infectious dose, nose or mouth breathing, tidal volume, breathing rate and timing so that an inspiratory breath in the susceptible contact occurs immediately after particle generation. So, whilst the basic concept of droplet transmission may at first be readily accepted, the constraining factors mentioned have actually led some to consider it a rare event *(39)*.

Aerosol Transmission

In this review, bioaerosols are defined as particles (droplet nuclei), typically ≤10μm in diameter, that carry a microorganism and are capable of both remaining suspended for long periods and travelling distances greater than 6ft. They can be generated by coughing, talking and breathing and may transmit infection on being respired into the respiratory tract. Gralton et al propose that the spread of infection by aerosolised particles is dependent on; the clinical manifestation of disease, the site of infection, the presence of pathogen and the type of pathogen *(37)*. The

process of disease transmission via aerosols has been reviewed in depth *(37, 38, 40)*. For influenza virus to be transmitted from human to human by the aerosol route it will need to be emitted from an infected individual in particles of a size range that can be respired by the exposed individual so bringing the virus into contact with target cells. In addition, the concentration of these particles must be high enough to cause exposure to an infectious dose. Furthermore, the virus will also have to survive the stresses of aerosolisation and be able to survive in the air for long enough to cause an infection. The behaviour of a virus within aerosol particles depends on the behaviour of the particle (aerosol physics) and the reaction of the virus to being in aerosol form (aerosol stability). These factors are now considered further along with evidence concerned with the detection of influenza in aerosols generated by infected individuals.

Aerosol Physics

The deposition of an aerosol particle is defined by Stokes law:

$$\text{Deposition Velocity } (u) = \frac{\rho d_p^2 g}{18\mu}$$

ρ- density of particle, μ- viscosity of air, g-gravity, d_p- particle diameter

Gravity and the viscosity of air can be treated as constants in an indoor environment, so the equation can be simplified to the settling velocity being directly proportional to the particles diameter squared and the density. Commonly the concept of aerodynamic particle diameter is used to define a particle of unit density as a further simplification. This is a useful concept as particle samplers are characterised on the basis of aerodynamic particle size. The larger the particle the quicker it will deposit from air
(Figure 2) and the less time that it is available for inhalation by an exposed person. Figure 3 shows the percentage of aerosol particles that can enter different sectors of the human respiratory tract; for a particle to enter the distal lung it needs to be very small (<5 microns for >10% deposition).

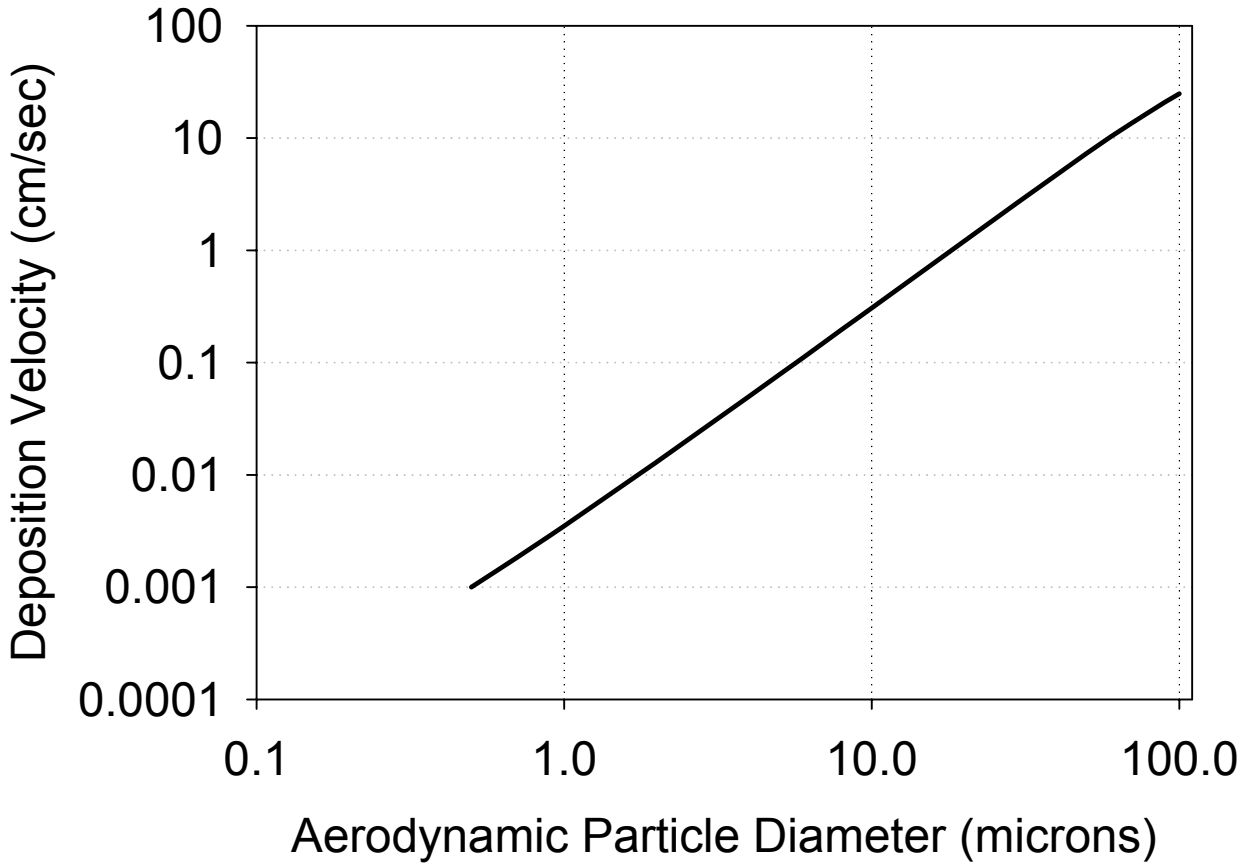

Figure 2; reproduced from *(41)*: Deposition of aerosol particles in still air against particle size.

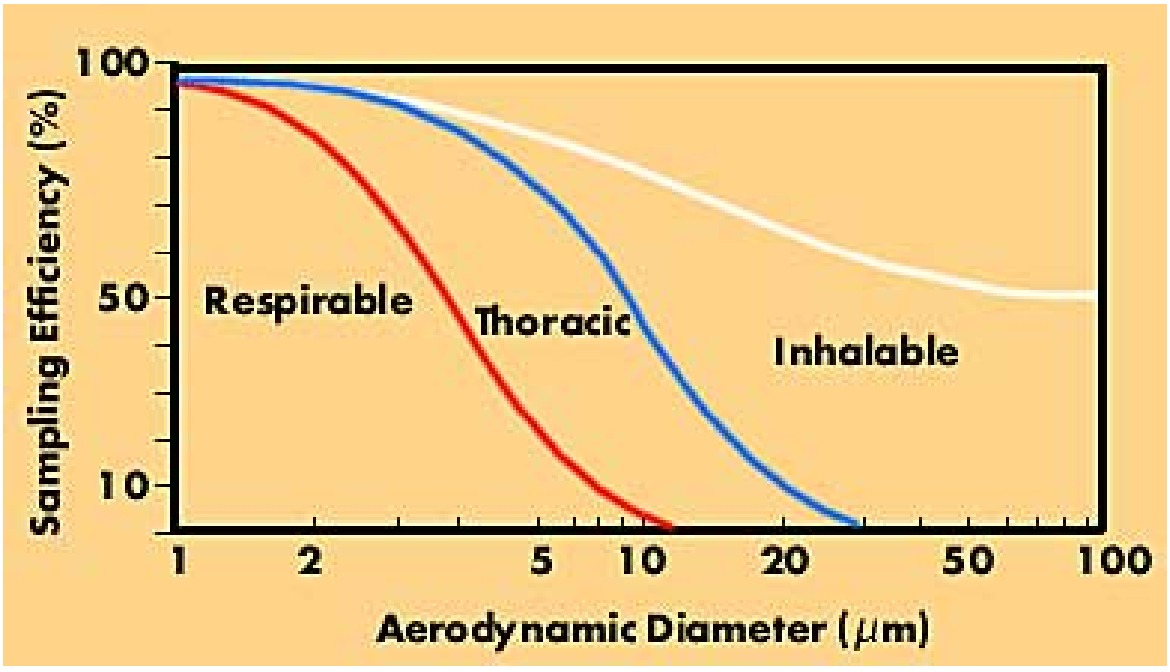

Figure 3; reproduced from*(41)*: The sampling efficiency of the human respiratory tract.

The most important factor determining whether influenza can transmit by the aerosol route is whether the virus is present in a large enough concentration. Data relevant to this includes:

- Chao et al *(42)* have studied the particle size distribution generated by a cough and show that 99% of virus would be expected to be in particles of >150 μm diameter when expelled from the respiratory tract.
- Lidwell *(43)* shows that the expected dried particle diameter is one fifth of the original diameter which means that 99% of virus would be present in nuclei of greater than 30μm.
- Using data from Chao et al, it can be shown (see appendix 1) that if patients have low titres of virus in respiratory secretions, virus will only be present in the larger particle sizes, but if patients are excreting higher titres then the presence of virus in low particle sizes is feasible. For example if the original titre is 10^7 $TCID_{50}$/ml then virus will be present in all particles ≥10μm, but only in only a fraction of particles <10μm when a patient coughs three times. Chao's data might also imply that aerosol transmission is more likely at the beginning of an influenza infection when patients typically excrete higher titres of virus than several days in to the illness, and that children might produce more aerosols as they excrete higher titres of virus than adults and for longer.

What does this mean for the aerosol transmission of influenza? Firstly, it shows that most of the particles produced by a cough will be large, deposit rapidly (99% at a velocity of greater than 3cm/second) and could only expose those close to the patient. Secondly, it shows that for infection to occur via aerosols in the distal lung, an infectious patient will have to have a high viral titre in their respiratory secretions (>10^7 $TCID_{50}$/ml). NB, this data is from individuals who coughed three times; if someone is exposed to more coughs then the potential for aerosol transmission will increase. A sneeze has not often been considered but is it worthy of mention as there are data to suggest that a sneeze produces a far greater (x100) bioaerosol load than does a cough *(44)*. Whilst it is generally held that coughing is a more common symptom of flu than sneezing *(45)*, data obtained during the 2009 pandemic shows that sneezing was common (93% vs 100% for cough) *(34)*.

Aerosol Survival

The production of an aerosol will inflict stress on a virus by desiccation, oxygen stress and UV effects. The persistence of virus in the air will be determined by its resistance to these

stresses. The material surrounding the virus will normally be protective but the concentration of potentially toxic components within this material such as salts maybe detrimental. Environmental factors such as relative humidity and temperature can also affect virus survival.

A number of authors have attempted to measure the survival of influenza virus in air (studies have been reviewed by Weber and Stilianakis *(17)*). Overall investigators find that survival is prolonged at low RH and this has lent support to the idea that low RH in indoor environments during winter time promotes virus survival and transmission. Using infection in mice as a detection Loosli et al reported maximal survival times of 1 hour at 80% relative humidity (RH) and 24 hours at 20% RH *(46)*. Mitchell and Guerin show large strain variation in aerosol stability between viruses obtained from different animals *(47)*. It is possible that differences in transmissibility of different influenza strains may in part be caused by their aerosol stability.

Methodological limitations to the reviewed studies should be noted. For example the size of aerosols used varied and the use of small particles (<3μm) may stress the virus to a higher degree than during natural generation which may lead to an underestimate of survival. In addition, many experiments were carried out in the dark, removing any potential impact of UV light.

Detecting aerosols produced by infected patients

Despite the above, the detection of live virus in aerosols, released into the natural environment by humans has not been shown before. Advances in technology have led to improved detection techniques such as PCR, but it remains that influenza can be difficult to work with and detect. Reasons for this include;

- Enveloped viruses such as influenza are sensitive to dehydration; this is influenced by temperature and humidity
- Viruses, especially RNA viruses, are sensitive to UV light
- Sufficient virus needs to be collected to enable culture. This is challenging because concentrations in air are often low and rapidly diluted in air as distance from the source increases
- Viruses are small and capture has required the use of filters which complicate recovery

- Whilst PCR allows great precision in identifying virus it does not tell us whether the recovered virus detected is viable (and therefore infectious)

The evolution of the materials and methods used to collect bioaerosols is contributing to progress in this field; a comprehensive review of methods was published in 2008 *(48)*. Contemporary efforts to detect influenza virus in aerosols have been successfully achieved by a number of groups, both in the laboratory *(49-51)* and from around patients *(34, 52-57)*.

- Fabian and colleagues have published work on the ability of four aerosol samplers to capture aerosolised influenza virus *(50)*. Crucially they used both molecular (PCR) and infectivity assays to detect virus, the latter demonstrating live virus. The samplers were; 1) a liquid impinger that could accommodate liquid collection media, 2) a cassette with a teflon filter, 3) a cassette with a gelatine filter and 4) a compact cascade impactor. All samplers collected virus detectable by PCR but the liquid impinger recovered live virus more effectively than the other samplers. The authors put this down largely to the effect of the liquid media assisting virus survival. They go on to say that new samplers are needed which employ liquid media to preserve infectivity. Fabian and colleagues also developed a technique to look for influenza virus in the exhaled breath of infected patients. Patients were asked to directly breathe into a device that collects filtered samples and employs optical particle counting and airflow data. Influenza was detected by PCR in 4 out of 13 samples collected from patients confirmed to be infected *(53)*.
- Blachere et al have described the use of a two stage, cyclone-based bioaerosol sampler. Following aerosolisation of influenza virus they were able to successfully collect and detect virus (by PCR). At the same time collected particles were size fractionated allowing particles of a respirable size to be identified *(49)*. They went on to test the samplers, which can be worn by individuals, in medical care facilities in the U.S. *(52, 54)*. Both stationary and personal samplers collected air particles containing influenza A virus; they were also successful in detecting Influenza B and rhinovirus particles in a second study. This sampler has also been used to collect air around individual patients with influenza (both in hospital and in patient homes); again influenza could be detected by PCR in these samples *(34)*
- Particle production from individuals with influenza has been assessed. Milton et al collected exhaled particles (≥5μm and 0.05 to <5μm) from 37 volunteers with seasonal influenza using a specially designed collection device. PCR and culture were used to detect virus. Virus numbers decreased rapidly between day one and day two of illness. Virus was

detected by culture from two subjects *(56)*. Lindsley et al collected cough particles from 47 volunteers with influenza; influenza was detected by PCR from 38 (81%) volunteers, 65% of the particles collected by samplers that could size fractionate were ≤4μm in diameter and viable virus was isolated from 2/21 samples tested *(55)*. As one might expect they found that the amount of virus detected (by PCR) from nasopharyngeal swabs correlated well with the amount of virus found during coughing, but they also revealed significant heterogeneity between individuals in the amount of virus detected during coughing (see below).

Another obstacle to understanding the nuances of aerosol production is that there exists significant variation both between individuals and even within an individual (when production over time will decrease). Healthy individuals differ in the numbers of particles produced during breathing, coughing, sneezing and talking *(58-61)*. The concept of super-spreading, transmission of directly transmitted infections (e.g. influenza, SARS) to an unusually large numbers of secondary cases from a source case, was introduced by Lloyd-Smith et al *(62)*; they present an analysis to show that the distribution of infectiousness is often highly skewed. With regard to aerosol transmission, differences can arise from a number of factors;

- Host factors = contact rates, behaviour/activities of host, viral shedding, symptoms, aerosol production
- Environmental factors = closed/open space, temperature, humidity

An outbreak that occurred on an aeroplane with no ventilation (discussed in more detail on page 31) where a high attack rate was observed *(63)* illustrates the point as do findings from a study that looked at social contact networks in young people *(64)*.

Discussion

The vast majority of virus released from an infected person during a cough or a sneeze is carried by droplets but despite their high infectious potential droplets face two major challenges; i) to reach their target cells and ii) to satisfy the relatively high infectious dose needed to initiate infection in the URT (compared to the LRT) and the assumption that droplet transmission is dominant is being challenged.

Aerosol science tells us that respirable infectious particles (≤10μm) can be produced by patients and that virus can remain viable (and therefore infectious) in these particles long enough to permit infection transmission. However, it should be appreciated that the risk of

infection may vary. For example a super-spreading patient who emits a large bioaerosol load into a contained indoor environment during winter time may represent a much higher aerosol infectious risk than that posed by a patient emitting a low bioaerosol load occupying a well ventilated room during summer. Viral shedding (a measure of viral load) is a different variable and again exhibits variation. For example we know that viral loads are higher early in the course of infection *(65-67)*, in children (compared to adults) *(68)* and in immunosuppressed patients *(69)*. Whilst high viral shedding could increase the risks of transmission by all routes, the potential for aerosol transmission may depend on it. An interesting hypothesis yet to be adequately addressed suggests that differences in virus inactivation on surfaces and in air caused by environmental conditions (e.g. humidity, temperature) may lead to changes in transmission pathways *(70-72)*.

Technology is moving forwards, only recently has influenza has been detected in aerosols (by PCR). Whilst this is important, it remains that viable influenza has only been isolated from aerosols produced by patients on four occasions and that the infectious dose needed to transmit infection via aerosols is unknown. However, a lack of data caused by technical challenges in experimental methods does not preclude the aerosol route being active; indeed it is almost certain that studies to date underestimate the amount of viable virus released.

To counter the risk of aerosol transmission several strategies have been proposed; they include use of UV light (to inactivate airborne virus) *(73)*, giving saline nebulisers to patients to decrease aerosol production *(61)*, wearing of respiratory protective equipment (e.g. a respirator) by carers and engineering controls such as room ventilation *(74)*. Gralton et al argue that as droplets and aerosols are produced simultaneously from an infected patient, any infection control precautions should protect against both modes, i.e. that droplet precautions alone may not suffice *(37)*.

Are the Proposed Routes of Transmission Plausible?

- The evidence base suggests that influenza virus can remain viable on surfaces and hands for periods which are consistent with onwards transmission
- There is good evidence that humans infected with influenza produce respiratory droplets and aerosols which contain influenza virus and are therefore of infectious potential

2. Outbreak Investigations

An outbreak can be defined as a time limited, temporary increase in the incidence of an infectious disease over and above baseline levels. It could concern the introduction of a 'new' infection e.g. pandemic influenza or a rise in an infection already known to exist in a population e.g. tuberculosis. The population might be small and localised e.g. patients on a hospital ward or it might include many people across entire countries. The primary function of an outbreak investigation is to instigate control and prevention measures, though the investigation itself can lead to opportunities to gain additional knowledge about the disease; for example spectrum of illness, transmission characteristics and incubation periods. The findings and dissemination of reports that concerned the recent influenza pandemic were very important in building a picture of the disease and allowed control and prevention measures to be refined.

Descriptions and investigations of influenza outbreaks can provide insights into the modes of spread that may have operated, though it must be remembered that they are not performed primarily for this purpose. Many influenza outbreak reports appear in the medical literature but relatively few are able to shed light on the precise routes of transmission that may have existed. For this to occur circumstances need to exist that allow a particular mode of transmission to predominate, whilst other factors remain constant. However, an outbreak by its very nature presents an uncontrolled situation which makes it very difficult to draw firm conclusions about what has occurred.

This chapter examines the evidence for influenza transmission that is provided by outbreak investigations and includes studies that concern the 2009 pandemic H1N1 virus.

Search Methods

To allow some inference about routes of transmission that occur during an influenza outbreak, reports should meet the following requirements;

- Occur in a confined setting to limit confounding from imported 'community' infections
- Have laboratory confirmed cases of influenza in the patients involved

A PubMed search using the above criteria was undertaken on 22/10/10. The search terms 'influenza' AND 'outbreak' AND 'transmission' generated 1671 citations. Thirty-seven titles

were found to be appropriate for further review and the abstracts were read. Seventeen full articles were read, six were ultimately selected for discussion. In addition, expanded searches and personal collections generated five further articles that are also considered. What follows is a chronological review of studies that permit attempts to define the routes of transmission that may have occurred.

Reports

Blumenfeld (75):
A prospective observation study was set up in a New York hospital during the Asian influenza (H2N2) pandemic of 1957. A number of papers were produced one of which details observations made of an outbreak occurring within a single medical ward. The ward accommodated 29 patients and 33 healthcare workers (HCWs) (20 of whom had been vaccinated) during an 11 day period when cases of influenza were appearing (Figure 4). Within seven days of illness in the index case, 28 persons (45%; 14 patients and 14 HCWs) had developed an influenza-like illness and 71% had serologic evidence of influenza infection. A further eight persons had rises in antibody titres but were asymptomatic. This gives an overall attack rate (AR) of 58%.

Cases seemed to occur in clusters suggesting close proximity was important in the spread of infection. It was also the case that infection occurred throughout the ward (apart from the side rooms which were not affected) raising the possibility of aerosol spread from the index case. The epidemic curve however, suggests that infection was propagated in the ward with HCWS being the most likely vectors (primary and/or secondary) of spread through close contact with patients. It is not stated what infection control precautions were used (if any) and whether ill HCWs continued to work.

It is difficult to rule out any route of infection transmission during this outbreak though there is little evidence to support long range transmission of infection.

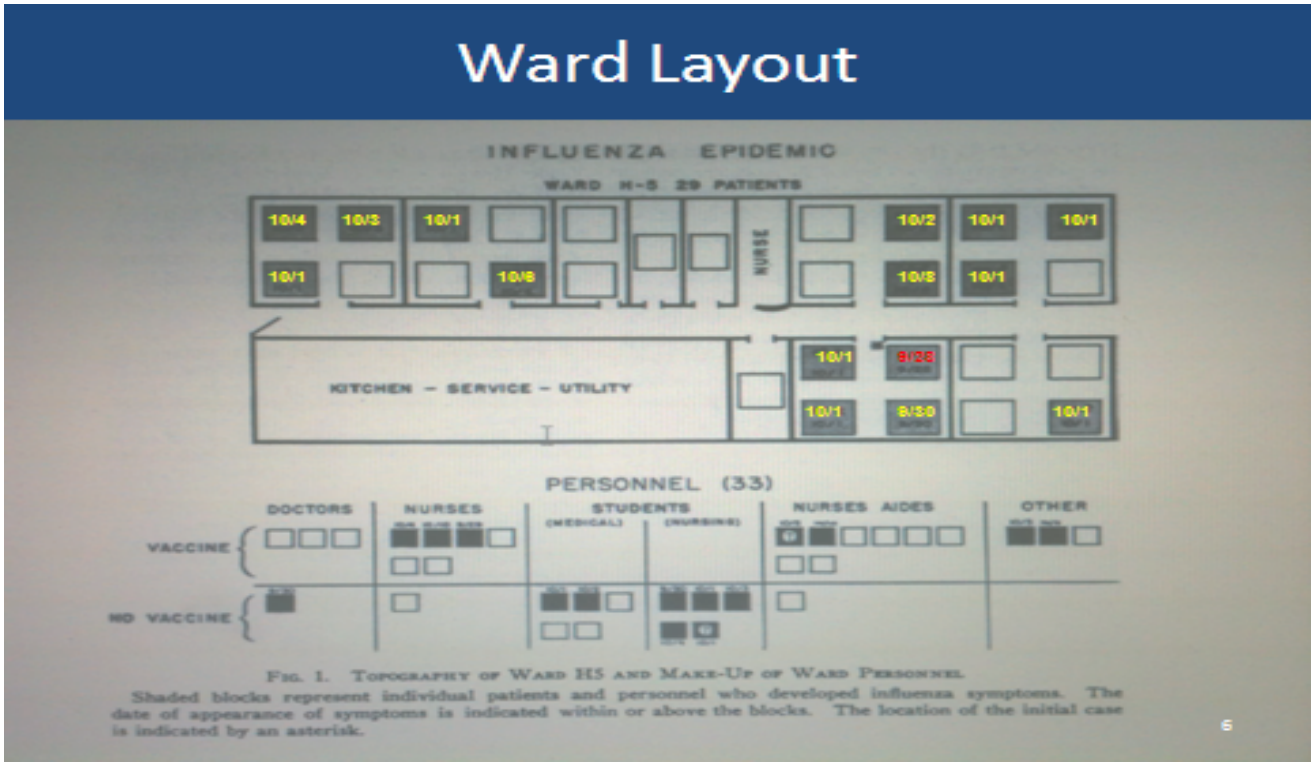

Figure 4; reproduced from *(75)*: Topography of ward and make up of ward personnel. Shaded blocks represent individual patients and personnel who developed influenza symptoms. The date of appearance of symptoms is indicated within or above blocks with the index case in red.

McLean(76):

Another prospective observational study took place during the 1957/58 pandemic in the US which centred on a hospital in California that had one building where UV lights had previously been installed. 150 patients resided in this unit, principally patients with tuberculosis and other chronic respiratory diseases. UV lights had been installed with the aim of disinfecting air of *M. tuberculosis*. Another hospital building housing 250 patients with respiratory disease but without UV light served as a control. Serological assessments were done before and after the second wave of the epidemic that struck in January 1958. Both clinical illness and serologically confirmed infection were reduced in the irradiated patient group.

Serologically confirmed AR;

- Irradiated = 2%
- Non-radiated = 19%
- Staff = 18%

These data do appear to make a compelling argument for effect of UV light on aerosols suggesting in turn that influenza is transmitted via aerosols (UV light cannot penetrate larger particles and will not affect virus associated with them). However, as the author acknowledges,

this was not a controlled experiment and several confounders may have existed. For example, no mention is made of patient movements, length of stays and there are no descriptions of cases. In addition, the environments on the two wards may have been different with respect to ventilation (e.g. airflows, open windows) and staff illness and movement between wards are not discussed in any detail. Because of these factors we cannot be sure that differences in attack rates seen were solely due to the effect of UV irradiation.

Moser (63, 77):
This report concerns an outbreak aboard a grounded aircraft that occurred in Alaska in 1977. A total of 54 people were on board the aircraft at some point during its four and a half hour grounding for a mechanical fault; 53 were followed up. The index case was a 21 year old female who was symptomatic with fever and cough. Although a throat culture was negative she was found to have seroconverted to an H3N2 influenza virus. Thirty passengers and crew stayed on the aircraft for the entire time, this includes the index case who lay across two seats and did not move about the plane. The exposure time for others varied as passengers were allowed to leave the aircraft and wait in the terminal building. In total 38 (72%) people became ill; 8/31 were culture positive and 20/22 were serologically positive. Individuals with greater than three hours of exposure had an AR of 86% whilst less than three hours of exposure gave a 54% AR.

This outbreak featured a single source of infection and gave a high AR. Importantly the aircraft presented a small and enclosed space. In addition the ventilation system had been switched off. The authors conclude that “exposure to large aerosols” was likely responsible for infection transmission. Large is not defined in this instance but it seems most likely that they are referring to aerosol spread as opposed to droplet. The index case could have been a super-producer of aerosols, which might help explain the very high AR. However, it is difficult to completely exclude the droplet and contact routes of infection; patients were able to move around the aircraft and thus close proximities to the index case could have occurred though it should be mentioned that the index case herself remained stationary and is not reported to have had direct contact with anyone else.

Morens (78):
The setting for this report was a nursing home residence in Hawaii, where an influenza (H3N2) outbreak occurred in 1989. Each of the homes 12 rooms contained one to four beds. Among

39 residents, 11 became clinically ill (28%); of these five were bedfast (11 were bedfast in total); six were virologically confirmed to have influenza and six died (three of whom were virologically confirmed). Infection control measures were instituted but only after more than two weeks from the onset of symptoms in the first case. An outbreak investigation revealed the following attack rates in residents;

- Those needing skilled nursing care 34% v those needing intermediate care 10%
- Bedfast 45% v non bedfast 21%
- Tube fed or frequently suctioned 38% v others 13%
- Those who were mobile and socialised with other residents had lower AR than bedfast or tube fed patients.

In addition, it was noted that individual staff were in contact with more nursing home residents than was normal.

The authors commented that the 'spatial and temporal patterns of onset not typical of airborne spread' and that 'we suspect staff spread virus by hands or fomites'. No staff illness was reported so it is unlikely that they acted as primary vectors. It is impossible to exclude either droplet or aerosol spread in the scenario described but the unusually high levels of patient contact and a lack of strict infection control procedures do appear significant.

Klontz (79):
The index case in this outbreak was a HCW employed at a naval base medical centre in Florida. It is likely that the HCW transmitted infection (influenza H1N1) to a squadron member who then accompanied colleagues on a 12 day assignment that involved air travel and accommodation in barracks. In total 59 secondary cases were identified including 41 squadron members.

Fourteen people became ill during the time away. Enlisted personnel (who shared accommodation) were found to have an eight times higher risk of infection than officers (who had private rooms). Ventilation in the barracks was provided by an air conditioning unit which provided re-circulated air to each room (Figure 5).

The authors assert that "barracks rooms with more than one case patient, supports the usual direct person-person mode of transmission by aerosolised droplets". The terms used here by the authors to describe routes of transmission are rather ambiguous. The findings suggest that

close proximity is important though this would allow all transmission routes to operate. It would appear that the ventilation system in the barracks did not facilitate long range transmission.

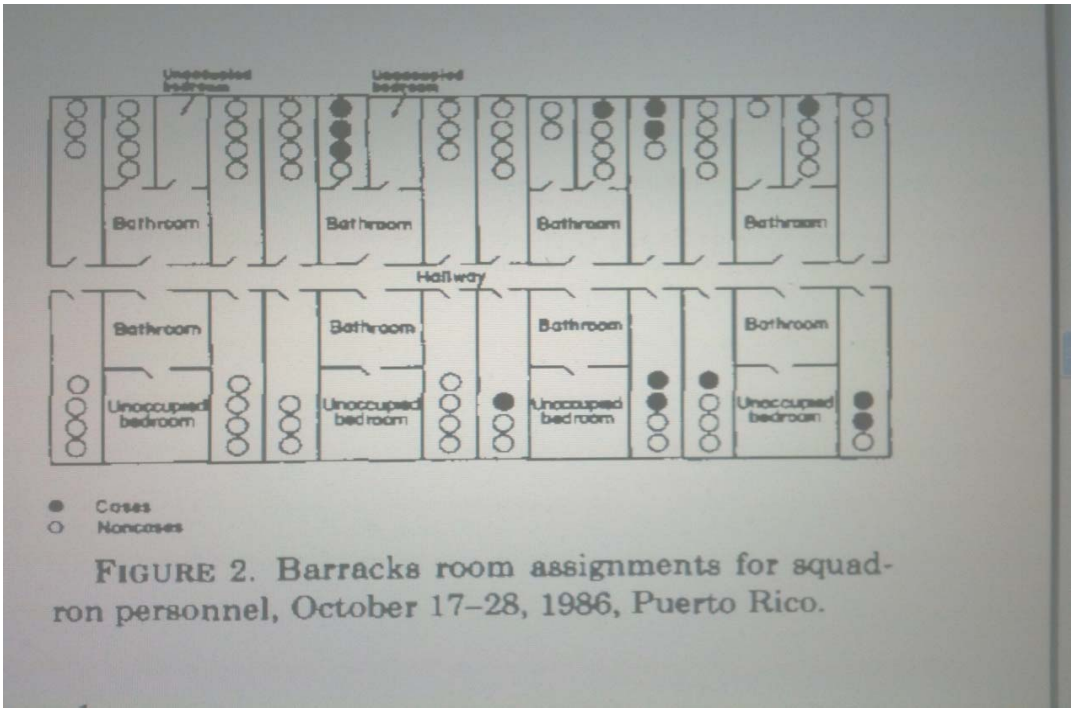

Figure 5; reproduced from *(79)*: Ventilation in the barracks was provided by an air conditioning unit which provided re-circulated air to each room. One of two barracks is shown above

Two aircraft were used to fly the 90 personnel home. Eleven individuals reported active symptoms (including coughing) during the flights; each flight lasted two and a half hours. Within 72 hours of return a further 23 personnel reported illness. Eight ill personnel were aboard aircraft number one, the risk of illness following this flight was 53% compared to 12% on aircraft number two. The ventilation systems aboard both aircraft were functioning and designed to completely exchange the air in the passenger cabin every four minutes.

Aboard the aircraft no route of transmission can be excluded, the authors state that “true airborne and person –person spread could have occurred on the aircraft”. Two comments can be made about the ventilation on the aircraft; i) we can compare the ARs seen in these aircrafts with that reported by Moser; a functioning system would appear to be important and can reduce aerosol transmission; ii) if the aerosol route is significant however, why did ventilation not reduce in-flight transmission further? It seems likely that other routes of transmission were acting.

Cunney (80):
An outbreak of H3N2 influenza occurred on a neonatal unit during an epidemic in Ontario, Canada in 1998. Of 54 neonates present in the unit over 18 days, 19 (35%) were confirmed cases though only six were symptomatic. 16% of staff reported illness during outbreak. Risk factors for infection in neonates were being a twin (OR = 7) and being mechanically ventilated (OR = 6.2). The unit was very busy over this period with >100% bed occupancy. The risk factors above seem to indicate that close contact is important. Perhaps parents were responsible for passing infection between twins, and nurses who have increased contact with ventilated children, also acted as secondary vectors. In the report the authors comment that they discovered that “ventilator tubing was being changed in a manner that produced aerosols”. No more information about this is given but it makes the point that the aerosol route cannot be discounted.

Awofeso (81):
An outbreak in a secure correctional facility is reported. The index case was an inmate who had received a pre-symptomatic visitor (the visitor became unwell later on the same day that the visit took place). Twenty-two cases of influenza were suspected and nine secondary cases were confirmed. Infection was confirmed in 35% of inmates, 13% of HCW and 0% of prison officers. It was suggested that inmates were infected through close contact with each other after contact patterns were traced and indeed the differences in attack rates for inmates and staff would seem to support this. The authors then go on to speculate that one HCW was infected after handling soiled linen, one inhaled virus containing droplets and one through face to face contact with inmate, though there appears to be little firm evidence of which to base these suppositions. Whilst close proximity to cases seems important, no routes of transmission can be considered to have occurred to a lesser degree than others.

Han (82):
A tour group comprising of 30 people travelled to China for a four day trip in June 2010. The index case was a female tourist who developed symptoms one day into the trip. Opportunities for transmission were identified as the tour itself that included time spent on a tour bus and two aeroplane flights. Confirmed secondary cases of H1N1 (2009) included nine tour group members and one aeroplane passenger who was not part of the tour group (this passenger was seated within two rows of the index case). The investigators reported that talking to the index cased for greater than two minutes (at a distance of less than 2m) was associated with

an AR of 56%; nobody who did not talk to the index case became ill. Furthermore, talking for greater than ten minutes increased the chances of becoming ill by five times compared to talking for between two and nine minutes.

It seems that close proximity to the index case was necessary for transmission, droplet or contact transmission are certainly possible. The authors state that there was “no evidence of airborne transmission”. Certainly, there is little evidence for long range transmission but the possibility of short range aerosol transmission (SRAT) is overlooked, especially when one considers that talking and even normal breathing can generate aerosols *(61)*.

Baker (83):
This retrospective cohort study concerns a school group (n=24) that travelled to Mexico and returned home on a flight from Los Angeles to New Zealand. A general practitioner in NZ identified cases of ILI amongst members of the group soon after their return and this led to an investigation to assess disease transmission during the air flight home.

During the flight 12 cases reported symptoms; nine were confirmed with H1N1 (2009), three were suspected. A post-flight case was defined as illness appearing within 3.2 days of the airplane landing. At risk for in-flight infection were 102 passengers in rear section of plane; 97 (95%) of these individuals were contacted and nasopharyngeal swabs were collected from 26.

Four post-flight cases were identified; of these two were deemed probable, one possible and one inconclusive for in-flight infection. The overall risk of infection in the rear section of the plane was 1.9%. For 57 passengers who were seated within two rows of a symptomatic case the risk was 3.5%. The authors conclude that the “mode of transmission cannot be established, all are possible including SRAT”. Long range transmission was not evident.

Apisarnthanarak (84):
This report concerns an outbreak of H1N1 (2009) that occurred amongst HCWs on a coronary care unit. The unit has the capacity for eight patients (including two isolation rooms) and employs 22 HCWs. The index case was admitted with an exacerbation of congestive cardiac failure that required ventilatory support. The diagnosis of influenza was made 48 hours after admission and at this time he was moved to an isolation room.

Seven HCWs (32%) and one patient were confirmed as secondary cases. All infected staff performed direct care for the index. Eight HCWs who administered care within 1m but did not have direct contact were not infected.

Contact transmission appears significant in this outbreak but a lack of detail about the exposure times of HCWs (e.g. did the none direct contact HCWs spend any less time with the index case than the HCWs performing direct care?) does not allow firm conclusions to be drawn.

Wong (85):
An outbreak investigation from a hospital in Hong Kong in 2008 that paid special attention to airflows is described. The setting was a 30 bedded medical ward that housed 59 patients and 29 HCWs during the course of the outbreak. The ward was composed of three bays (A, B and C) and a side room (Figure 6). The index case had chronic obstructive pulmonary disease and received non-invasive ventilation for 16 hours on the ward (Bay C) beginning on March 31st. Influenza H3N2 was subsequently diagnosed. Nine inpatients were confirmed as secondary cases and two HCWs developed symptoms but were not virologically confirmed. All cases received oseltamivir within 24hrs. The overall patient AR was 13.6%; ARs in Bays C, B and A were 20%, 22.2% and 0% respectively. The risk of infection was found to be highest on 31st March and 1st April.

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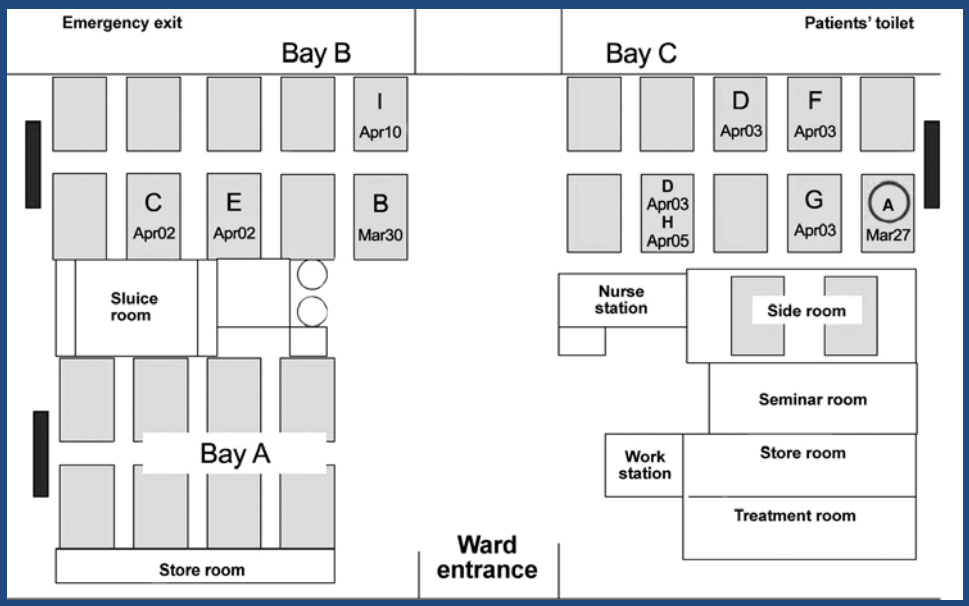

Figure 6; reproduced from *(85)*: Layout of the outbreak ward and the locations of affected patients. Patient A (circled) was the index case. Dark-colored blocks represent high-efficiency particulate absorbing (HEPA) filters placed at the wall end of each ward bay. Dates of symptom onset were stated for all infected patients. Patient D had been staying at two bed locations (front row then back row).

A variety of devices were in operation that affected airflows on the ward; i) an air conditioning was provided by a system that had outlets at ceiling level in each bay. Return air grills were located in the ward corridors; ii) air purifiers were also located in each bay; in bays A and B the fan setting was low but in Bay C the fan setting was on medium. This resulted in a net flow of air from Bay C into the corridor and towards Bay B (Figure 7).

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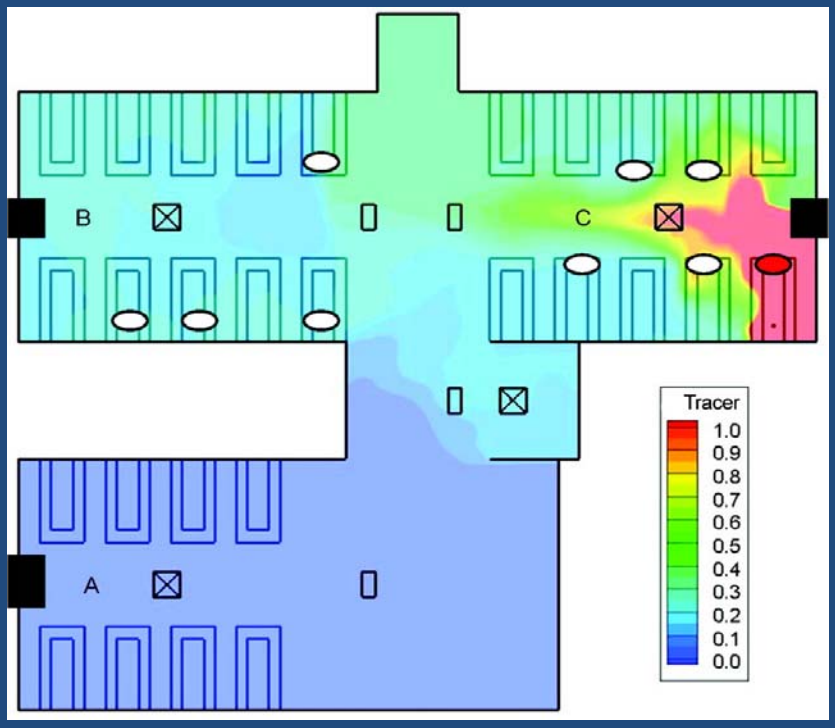

Figure 7; reproduced from *(85)*: The spatial distribution of normalized concentration of hypothetical virus-laden aerosols (modeled as gaseous tracer) in the outbreak ward at a height of 1.1 m. All high-efficiency particulate absorbing (HEPA) filters were assumed to function with 100% filtration of the modeled droplet nuclei. The 3 HEPA air purifiers are shown as black boxes, the 4 diffusers are shown by a square with an X, and the 4 returns are shown as a small rectangular filled box. Affected patients are represented by white ovals (the index patient is marked as a red oval).

The outbreak was temporally related to an aerosol generating procedure involving the index case and imbalanced airflow on the ward. The authors state that droplet and contact spread cannot entirely explain all instances of infection transmission. They cite as evidence the epidemic curve which supports a point source for the outbreak, the spatial distribution of secondary cases seen and the fact that close contact transmission was minimal as there was little patient interaction and little evidence that HCWs acted as vectors. This study presents a unique set of circumstances and convincing evidence for the presence of aerosol transmission.

Author	Setting	Virus (year)	Special features / identified risks	Author conclusions re route(s) of transmission	Reviewer conclusions re dominant route(s) of transmission
Blumenfeld	Hospital ward	H2N2 (1957)	Pandemic virus	-	All routes possible
McLean	Hospital Ward	H2N2 (1957)	UV light, pandemic virus	Aerosol	Aerosol
Moser	Aircraft	H3N2 (1977)	Point source, no ventilation	Aerosol	Aerosol
Klontz	Barracks and aircraft	H1N1 (1986)	Outbreak amongst a squadron	Droplet	All routes possible
Morens	Nursing Home	H3N2 (1989)	High level care patients	Contact	Contact
Cunney	Neonatal Unit	H3N2 (1998)	Twins, mechanical ventilation	Close contact	All routes possible
Awofeso	Prison	H3N2 (2000)	Infection introduced into a closed community	Contact / droplet	All routes possible
Han	Tour group + aircraft	H1N1 (2009)	Talking with index case, pandemic virus	Contact / droplet	All routes possible
Baker	Aircraft	H1N1 (2009)	Pandemic virus	All routes possible	All routes possible
Apisarnthanarak	Hospital ward	H1N1 (2009)	HCW providing direct care, pandemic virus	Contact	Contact / Droplet
Wong	Hospital ward	H3N2 (2008)	Aerosol generating procedure and airflows	Aerosol	Aerosol

Table 2: Outbreak reports/investigations are presented with respect to setting, virus involved, special features and proposed routes of transmission.

Discussion

Many outbreaks are described and most concentrate on epidemiologic aspects; few are able to define precise situational occurrences with reference to an index case, environmental conditions and susceptible individuals. Outbreak reports are extremely heterogeneous, each relates to a specific situation with a variety of key factors (Table 2):

- Virus – different strains may have vary in infectiousness and transmissibility
- Human hosts – differences in immunity and social and physical behaviour will exist, the presence of super-spreaders *(61)* and asymptomatic cases in a population will be important
- Environment – the setting, temperature, humidity and airflows will vary between outbreaks
- Contact Tracing - this is often incomplete and virological confirmation of cases is difficult to achieve

Furthermore, definitions used and interpretation of data can vary between authors.

The very nature of an outbreak means that conditions are not formally controlled in any way making it very difficult to draw firm conclusions about specific risk factors for, and routes of transmission. This leaves us to interpret findings based upon observations only. Some studies do describe situations akin to a control and intervention group, whilst others describe specific environmental factors that existed which may have influenced the spread of infection.

Repeated observations of outbreaks in closed settings show that as population densities increase, attack rates also increase. This implies that short range transmission, by whatever route, is key. Long range transmission is a rare event; however, this does not mean that aerosol transmission can be discounted. The concept of SRAT is ignored by many authors with the consequence that transmissions that have been seen to occur through close contact are put down to either droplet or direct contact spread. In the studies reviewed no routes of transmission can be completely excluded, circumstances related to the environment and individuals involved will dictate which route(s) predominate. For example, the reports by Moser, Mclean and Wong all show that aerosol transmission likely occurred because of circumstances that favoured this route.

Evidence from Outbreak Reports

- In outbreak studies, no routes of transmission can be completely excluded
- The data do not offer any clear consensus about the relative importance of different modes of transmission as the circumstances, environmental conditions, and patient characteristics are highly heterogeneous
- It is difficult to distinguish which routes of infection have been active during close 'contact' between infected and susceptible individuals. However, the circumstances described in several of the reports do not fully exclude a role for aerosol spread
- Nevertheless, long-range transmission is rarely reported and is unlikely to be important

3. Prospective intervention studies in the setting of natural infection

Non-pharmaceutical interventions (NPI) such and hand hygiene (HH) and face masks are recognised by WHO as being potentially useful to reduce the transmission of influenza between people *(86)*. Such interventions may be able to tell us something about transmission routes because they act by disrupting one or more of them. For example if HH is shown to reduce illness rates then it implies that the contact route of transmission is significant and if wearing a surgical face mask (SFM) reduces illness rates then either the contact and/or droplet route(s) are important (a SFM will act as a barrier to both).

Search Methods

A variety of studies that have assessed both pharmaceutical and NPI have taken place. They have been conducted in both community and hospital settings, some have looked at single interventions whilst others have been randomised controlled trials assessing multiple interventions. These studies or reviews of them will now be discussed but the scope is limited to the information they can give about routes of transmission.

The authors own reference collection, relevant review papers and bibliographic searches of selected articles were used to identify studies. In addition, PubMed searches looking for related citations to those already selected were performed.

Studies

Hand Hygiene

Three systematic reviews *(87-89)* and one meta-analysis *(90)* that included data on HH to reduce the spread of acute respiratory infections (ARIs) have been conducted. One review was specific to influenza *(87)*, but in general these papers relate to acute respiratory infections as a whole as there is little data which is organism specific. All reviews comment on the heterogeneity and often poor quality of studies done, but all conclude that HH can reduce episodes of respiratory illness. Two papers report pooled estimates of effect of 16 and 21% *(89, 90)*.

A study assessing the impact of a HH campaign on the incidence of laboratory confirmed influenza and absenteeism has recently reported findings. The trial, conducted in Egypt, introduced an intensive HH programme to 30 schools over a 12 week period; 30 different schools acted as controls. In the control arm there were 0.5 episodes per 100 student weeks of absence due to an influenza-like illness (ILI), in the intervention arm the rate was 0.3. This gives a risk reduction of 40% ($p<0.0001$). The incidence of laboratory confirmed influenza (both A and B) between the control and intervention group was also significantly reduced *(91)*.

Face masks

A systematic review of the evidence that face masks can prevent influenza transmission was undertaken by Cowling et al *(92)*. It concluded that there is some evidence to support the use of either a SFM or respirator by an infected person to protect others but fewer data to endorse the wearing of a SFM to prevent the wearer from becoming infected. However, it should be recognised that the evidence base is small and quality of the studies reviewed was variable. Four of the studies included in the review are discussed in sections that follow.

Using the schlieren optical method to visualise airflows around human subjects, Tang et al show that a cough projects a turbulent jet into surrounding air and that this can be blocked by wearing a respirator or redirected by wearing a SFM *(93)* (Figure 8). More recently, Milton et al have shown that SFMs worn by influenza infected subjects can reduce the number of virus containing particles emitted. Larger virus containing particles (≥5μm) were reduced more than smaller particles (<5μm); overall SFMs produced a fivefold reduction in viral aerosol shedding *(56)*.

Figure 8; reproduced from *(93)*: Schlieren images of two volunteers facing one another. The subject on the right is masked as a precaution. The volunteer on the left coughs in the direction of the other subject first without wearing a mask (a) (the cough plume can be seen directed downwards at roughly a 30° angle), then while wearing a standard surgical mask (b), and finally while wearing an N95 mask (c). The different behaviours of these coughs are sketched in (d).

Two large randomised studies have reported data on the use of face masks to reduce influenza transmission by studying nosocomial transmission between patients (naturally infected) and healthcare workers who attend them.

- The objective of Loeb's study was to compare SFMs with respirators (FFP2/N95) to protect healthcare workers from influenza *(94)*. Nurses working in Canadian emergency departments were randomised to a mask and asked to wear it whilst caring for patients with febrile respiratory illnesses during an influenza season. 446 nurses were recruited and the primary outcome was laboratory confirmed (PCR and/or serology) influenza. Influenza was diagnosed in 50 (23.6%) of nurses in the SFM group and 48 (22.9%) of nurses in the respirator group (absolute risk difference, −0.73%; 95% CI, −8.8% to 7.3%; p=0.86), indicating no significant different between outcomes in the two arms. The vast majority of influenza diagnoses were made by serology; ILI was reported by only 11 nurses (nine in the SFM group and two in the respirator group, a non-significant difference) suggesting that the study was markedly under-powered for ILI and PCR based endpoints.

- Macintyre's study also compared SFMs with respirators (FFP2/N95) to protect healthcare workers from ARIs (note - full study findings have yet to be published). 1,936 emergency department and respiratory ward nurses and doctors were recruited across 24 hospitals in Beijing, China. A non-randomized comparator group were asked to continue with 'usual practice' whilst other recruits were cluster randomized to one of three intervention arms; i) SFMs, ii) fit-tested N95 respirators, iii) non-fit tested N95 respirators. Masks were worn during all work hours for four consecutive weeks. Initial findings revealed that consistent SFM use was no better than usual practice for prevention of clinical respiratory illness or ILI, whilst N95 respirators significantly reduced infection rates *(95)*. However, the data has since been re-analysed (following removal of the non-randomized comparator arm) and clinical infection rates between the mask groups are no longer statistically significant though the author states that respirators performed better *(96)*.

Until the final version of MacIntyre's paper is published it is unclear to what extent these studies have produced conflicting results. An intrinsic limitation of both studies is that the relative risk of transmission within the study context (the hospital) and outside (i.e. the household and community) is unknown; if most exposure of healthcare workers occurs outside the healthcare context, such studies will always be limited in their ability to demonstrate a significant difference in intervention effectiveness even if one occurred.

<u>Community intervention studies</u>

Prospective community studies have enrolled participants (individuals, families, households) into randomised intervention trials, often following the identification of an index case, and followed during a period of influenza activity. Participants are assigned to interventions such as HH or face mask use to reduce transmission. Five major community studies have been conducted:

1. Aiello et al - Primary prevention study *(97)*:
   1372 young adult residents in university accommodation were assigned to SFM use, SFM plus HH or a control arm for six weeks during an influenza season (06/07) in the US. 368 (32%) subjects subsequently reported symptoms of ILI and 94 samples were obtained for virological analysis. Ten of these were positive for influenza, i.e. 3.7% of ILI was confirmed as influenza. Neither intervention resulted in a significant reduction in cumulative ILI

incidence over the entire study period but during weeks four to six there was a significant reduction of 35% (95%CI, 9%–53%) to 51% (95%CI, 13%–73%) in ILI in the SFM plus HH group and during weeks four and five there was a significant reduction in ILI of 28% (95%CI, 2-47%) to 35% (95%CI, 2-57%) in the SFM only group. It is worth noting that the average use of a SFM each day was only three and a half hours. While the authors suggest SFMs had the largest impact in transmission reduction, it is important to note that 'normal' hand washing continued to take place in all study arms as it was use of a specific hand sanitizer that was being assessed. The study was unable to address the issue of how SFMs reduced transmission; i.e. were masks blocking the release of respiratory secretions from infected wearers, or were they protecting uninfected individuals by acting as a barrier or a no-touch-face behaviour modifier?

2. Cowling et al - Secondary prevention study *(98)*:
   Index cases presented for medical care within 48 hours of symptom onset and tested positive for an influenza rapid antigen test. The household of the index case was then randomized to interventions to reduce transmission. Interventions were i) control, ii) HH and iii) HH plus SFM. 259 households (794 individuals) were subsequently visited and samples collected for viral testing; the primary outcome was laboratory confirmed influenza in household contacts. Adherence to interventions varied and contamination between groups occurred. Less than half of the index patients in the SFM plus HH group reported regular use of a SFM during follow-up and adherence among household contacts was lower. Good adherence to the HH intervention was no better than 62% in any group. The secondary attack rate in the study was low (8%) and no differences were seen across the intervention arms. In a subgroup of households who implemented the interventions within 36 hours of symptom onset, transmission was significantly reduced (adjusted odds ratio, 0.33 [95% CI, 0.13 to 0.87]) in the HH plus SFM group.

3. MacIntyre et al - Secondary prevention study *(99)*:
   286 adults from 143 households where a child was unwell with a respiratory illness were recruited (influenza was detected in 21% of children). They were randomised to interventions that consisted of i) SFM, ii) respirator (FFP2/N95 mask, not fit tested) and iii) control. ILI was reported in 16%, 22% and 15% of adults in each group respectively; there were no statistically significant differences. Good compliance with mask use, defined as 'wore mask most or all of the time' over a five day period was reported by 21%. In a

subgroup of adults who were adherent, use of either mask reduced their risk for ILI by between 60-80%.

4. Larson et al – Secondary prevention study *(100)*:
   509 households were randomised to i) education, ii) education and HH or iii) education, HH and SFM and followed up for 19 months. 5034 upper respiratory tract infections (URTIs) were recorded; 669 were consistent with an ILI and 78 were laboratory confirmed as influenza. In multivariate analyses no additional benefit of HH or SFM over education was seen on the overall incidence of URTI. Despite poor compliance mask wearing was associated with reduced secondary transmission of URTI as was (rather counter intuitively) increased household crowding and index cases who were less than five years old.

5. Suntarattiwong et al – secondary prevention study *(101)*:
   Households were randomised to i) control, ii) HH or iii) HH and SFM use. The final analysis included 348 households (887 members). All index cases were ≤ 15years old (46% were ≤5 years old); they were infected with various influenza types (H1N1 = 14%, H3N2 = 32%, Flu B = 16%, pandemic H1N1 = 38%), and notably, 92% slept in the room same as their parents. They observed a high SAR (18%) compared to other studies. At both the individual and household level the interventions did not protect against secondary infections even if instituted within 48 hours of symptom onset in the index case;
   - Individual level: HH OR = 0.97 (95% CI 0.57-1.64), HH+SFM OR = 1.34 (95% CI 0.78 -2.30).
   - Household level: HH OR = 1.1 (95% CI 0.75-1.61), HH+SFM OR = 1.23 (95% CI 0.85 -1.78).

There are indications that some of interventions deployed in these challenging studies may have had some benefit in certain situations, but none showed any positive results with regard to stated primary objectives. Compliance with interventions use was a particular issue, as is the fact that the interventions in the secondary prevention studies are often only deployed once symptoms begin and so miss periods of possible transmission when an index case is asymptomatic. The difficulties and limitations faced by these community studies are outlined in Table 3.

Difficulties and limitations of inferring modes of transmission of influenza from community studies
Although able to generate some data on the effectiveness of interventions, these studies are unable to reveal which route(s) of transmission have been reduced; • A respirator could reduce hand-to-face contact, droplet and aerosol exposure - which is most important?
The number of participants required and therefore the costs involved are considerable given the low clinical attack rates of influenza seen in recent seasons and the potentially modest effect size.
Use of clinical case definitions alone to identify patients is problematic; • Results from English GP-based sentinel virological surveillance in 2008/09 show that of 34% of samples taken from patients who present with an ILI are positive for influenza *(102)*. In the US over recent years, the percentage of respiratory samples that test positive for influenza during an influenza season is <20% *(103)*
Most studies rely on PCR based identification of influenza virus from nose and throat specimens to assess outcomes. • The ideal specimen is a nasopharyngeal aspirate *(104-106)* but this is often considered overly invasive in a community setting. Furthermore, viral shedding varies by day of illness so studies ideally need to sample early in disease and at multiple time points in both index cases and contacts.
Studies based on a mixture of ARIs are able to generate more power, but have to assume that the contributions of different modes of transmission are the same for all respiratory viruses. Given the available data on influenza, RSV and rhinovirus transmission this is probably a false assumption *(107)*
A subject's compliance with study interventions e.g. face mask use and hand hygiene, is often low and this has proved to be a major obstacle. Compliance may be much higher in a pandemic because of perceived risk, but this is difficult to simulate for 'normal' seasonal influenza.
Confounding variables are difficult to eliminate in community infection studies. Although in theory randomised controlled trials eliminate confounding this is only the case if intention to treat analyses are used. To date no studies have demonstrated an effect of the interventions using an intention to treat analysis and have needed to resort to subgroup analyses based on uptake of the intervention.

Table 3: Difficulties and limitations of community studies.

Discussion

Regular HH reduces the incidence of ARI, but the majority of studies are not specific to influenza and it is debatable whether rhinovirus and RSV for example transmit in the same way. However, this evidence, combined with that from the study by Talaat et al, does suggest that the contact route of transmission maybe significant.

With regard to face masks, the majority of studies show some evidence of effect though it is difficult to say how this effect is mediated e.g. through reduced face touching or as a physical barrier to droplets. Furthermore, the beneficial effects of face masks are often seen in combination with HH interventions. Findings from the Loeb and MacIntyre (household) studies might imply that aerosol spread is of limited importance because respirators afforded no extra protection beyond other existing control measures. Interpreting MacIntyre's HCW study as suggesting respirators have somewhat greater effectiveness than SFMs might indicate a more significant role for aerosol transmission.

Comparing the two papers by MacIntyre is interesting; was wearing a respirator more effective in the hospital setting because the risk of aerosol transmission in a hospital is higher than in the community? As a result of clustering a number of infected patients into one area and the possible occurrence of aerosol generating procedures (e.g. chest physiotherapy, assisted ventilation), the bioaerosol load in a hospital may be higher than that in a household environment. However, it should be recognised that other important differences also existed, e.g. people studied (household member vs. professional HCW) and country setting (China vs. Australia).

A problem with using interventions to assess modes of transmission is that blocking one route still allows transmission to take place down an alternative route. For example if contact transmission is blocked by HH, transmission could still occur via droplets and aerosols. If it does, HH won't appear to be as effective as it really is. Competing risk style models are required to make accurate inferences about the routes of transmission involved. This illustrates the point that transmission can likely occur through multiple routes in the same patient; it is a dynamic and opportunistic process.

While the studies discussed have the potential to give an indication of the 'real world' efficacy of interventions, they are unable to provide the emphatic evidence sought by governments and policy makers, especially with regards to modes of transmission. Indeed, a recent discussion paper following a series of studies funded by CDC (including all five of the studies mentioned above), recognised ongoing evidence gaps to be "the relative contributions of influenza virus transmission modalities to disease spread" and "the efficacy of different types of masks, HH, and combinations of personal protective measures for reducing transmission of influenza" *(108)*.

Evidence from Intervention Studies

- Intervention studies designed to evaluate the effectiveness of specific items such as facemasks or respirators do not lend themselves to easy determination of the routes of transmission involved
- To date the balance of evidence from randomised studies suggests that respirators seem no more effective than SFM in preventing influenza transmission. Although this might suggest that the aerosol route of transmission is less significant than droplet, several other factors could have influenced study findings
- Randomised studies of HH are easier to interpret in relation to establishing the role of contact transmission (but not its relative importance compared with droplets and aerosols)

4. Human Challenge Studies

Experimental human challenge studies present an attractive way to study influenza transmission. Some of the earliest published human challenge experiments took place during the 1918/19 influenza pandemic when attempts were made to demonstrate the transmission of infection from symptomatic patients with presumed influenza to healthy volunteers *(109)*. These experiments were unsuccessful, probably because the volunteers were immune. A similar design today would be logistically complex (with a need to have immunologically naive volunteers ready and waiting), raise ethical issues in relation to subject safety and could cause the propagation of a nosocomial outbreak.

Experimental challenge studies have been used to investigate the transmission patterns of other respiratory viruses. Hall and Douglas concluded that RSV is transmitted predominantly through close contact and fomites *(110)*. Rhinovirus transmission has been studied extensively using challenge experiments (reviewed by Hendley *(111)*); transmission has been shown to occur through both contact (via hands and fomites) and large droplets and/or bioaerosols. However, in his review Hendley has difficulty drawing firm conclusions about routes of transmission because studies have not been able to isolate all the different transmission routes and often the study designs were somewhat contrived and as such did not reflect natural conditions. Couch et al conducted a series of challenge experiments involving coxsackievirus, adenovirus and rhinovirus concluding that both contact and airborne transmissions likely occur *(112)*.

Search methods

The authors own reference collection, relevant review papers and bibliographic searches of selected articles were used to identify studies involving influenza challenge where data informing aspects of transmission were obtained. In addition, PubMed searches looking for related citations to those already selected were performed.

Studies

The first successful influenza challenge study took place in 1936 when volunteers were infected with atomised suspensions of infected mouse lung *(113)*. In 1946 Henle published

findings from over 200 volunteer exposures and identified the route of inoculation as important; infection by inhalation led to fever much more frequently than did nasal instillation (89% vs. 13%) *(114)*. In addition, there is evidence to suggest that the infectious dose required for aerosol inoculation (0.6-3 $TCID_{50}$) *(115)* is substantially lower than that required for intranasal inoculation (100-1000 $TCID_{50}$) *(116-118)*. In Alford's study an H2N2 virus aerosol was produced using an atomiser which generated particles in the 1-3μm range. Twenty three volunteers (14 of whom had antibody titres to the challenge virus of ≤1:40) inhaled 10 litres of the aerosol which was delivered via a facemask. The dose of virus delivered ranged between 1-126 $TCID_{50}$, in the majority (14) the dose was <5 $TCID_{50}$. Four volunteers developed clinical illness; virus was isolated from these and one other volunteer, whilst seroconversion was seen in seven including all those who exhibited illness. Noting limitations of the study design and making an assumption that only 60% of the aerosol load inhaled will reach the lower respiratory tract the study reports that half of the volunteers with very low pre-existing antibody titres were infected with 0.3-6 $TCID_{50}$. In another study, Jao and Jackson inoculated 30 $TCID_{50}$ of the same virus to volunteers via intranasal spray; 12/30 (40%) became ill *(119)*.

In a study which attempted to compared natural and experimental influenza, it was found that natural infections produced more fever, more cough and had a more marked effect on pulmonary function tests *(120)*. Possible explanations for this lie in differences between the infecting viruses themselves and the route of inoculation.

In present-day influenza challenge studies, susceptible healthy adults are selected by serum antibody levels and infected intranasally with a well-characterized pool of wild-type influenza virus (the aerosol route of inoculation is not used as there is a concern that infections induced in this way may be more severe). Under these conditions, the majority of subjects will be infected and develop a mild ILI accompanied by recovery of virus from the nasopharynx. This model has been used to evaluate antiviral agents, including neuraminidase inhibitors (NI), and influenza vaccines *(66)*.

Findings from studies that have assessed the use of the NI zanamivir have contributed to evidence concerning sites for virus acquisition. Intranasal zanamivir has been shown to be useful as prophylaxis against an intranasal influenza challenge, however it was found to be ineffective for close or household contacts of natural influenza (odds ratio [OR], 0.90; 95% confidence interval [CI], 0.30-2.72; P=.855). It was also the case that neither inhaled zanamivir

or combined inhaled + intranasal zanamivir were proven to be effective though there was a trend towards protection (OR, 0.27; 95% CI, 0.07-1.05; P=.058 and OR, 0.52; 95% CI, 0.17-1.58; P=.247 respectively). In separate studies, inhaled zanamivir was shown to be effective as post exposure prophylaxis (efficacy 80%; 95% CI, 61-90; p<.001) and as seasonal prophylaxis (efficacy 67% CI, 39%-83%; P<.001). Finally in a study that compared inhaled +/- intranasal zanamivir with placebo in the treatment of uncomplicated influenza, the time to alleviation of major symptoms was one day shorter (four days vs. five days) in patients given inhaled and intranasal zanamivir (P=0.02) and in patients given inhaled zanamivir alone (P=0.05) than in patients given placebo. The addition of intranasal zanamivir did not seem to add clinical benefit (though the study was not designed to assess this) but it did reduce viral titres in the URT more quickly than did inhaled zanamivir alone.

Discussion

Experimental challenge studies have shown that infection by both intranasal inoculation and aerosol routes can cause infection and they suggest that infection via aerosols, initiated through cells in the LRT requires a lower infectious dose. These findings have led some to conclude that the LRT is the preferred site of infection and by implication (as only aerosols can reach it) that the aerosol route of transmission is important *(7, 120)*. In addition, studies seem to suggest that antiviral prophylaxis of the nose alone does not prevent natural influenza whereas orally inhaled zanamivir does, which points to the pharynx and/or tracheobronchial tree as key sites for virus acquisition. In terms of routes of transmission, this data does not allow us to discriminate between droplets and aerosols as both can reach the pharynx, but it does suggest that the contact route may not play a dominant role.

In analysing the findings from experimental challenge studies it should be recognised that that the inoculation methods employed are unlikely to accurately replicate transmission that occurs in natural settings. For example the size, concentration and viral load of aerosols and delivery methods that have been used to achieve infection by aerosols are rather artificial. This does make it difficult to relate the findings from experimental infections to what might be happening in natural circumstances.

Challenge studies could offer a promising approach to gain insights into both the mechanisms of influenza transmission and its prevention, so long as a reliable model of transmission can be developed. Whilst such an approach may not fully replicate infection transmission cycles in the community, some fundamental issues could nevertheless be addressed. A proof of concept study (funded by the Department of Health, England) has shown that influenza can be transmitted from experimentally infected volunteers to other susceptible volunteers (Dr B. Killingley – personal communication). If high attack rates can be generated in such studies then interventions can be used to assess routes of transmission.

Evidence from Human Challenge Studies

- Human challenge studies have not yet been exploited to evaluate modes of influenza transmission
- Challenge studies performed to investigate pathogenesis suggests that a smaller viral dose is needed to initiate infection in the lower compared to the upper respiratory tract
- However, the ability of experimentally induced infection to act as a surrogate for natural infection is not fully accepted
- Studies using antivirals administered via different routes also suggest that the lower respiratory tract is the preferred site for initiation of infection in humans; this might indicate the potential importance of aerosol transmission

5. Animal Studies

Animal studies have played an important role in advancing our knowledge about influenza and its management, indeed it was through the use of a ferret model of infection that influenza was first isolated by Smith and colleagues in 1933. Studies showed that throat washings obtained from humans, who had an influenza-like illness, could be used to infect ferrets and produce a very similar disease *(2)*. Studies seeking to improve our understanding of influenza transmission have often employed mice or ferrets. However, the murine model has fallen out of favour because researchers have experienced difficulties in getting the virus to transmit consistently between mice *(121, 122)* and the guinea pig has been proposed as an alternative *(121)*. Using these animals transmission factors related to the host, the environment and the virus itself have been explored. Studies that have attempted to investigate the specific routes of transmission in animal models and factors that affect them will now be reviewed.

Search Methods

A PubMed search was undertaken on 02/11/10. The search terms ‘influenza’ AND ‘transmission’, limited to animals, English and abstract available generated 1231 citations. Twenty-nine titles were found to be appropriate for further review and the abstracts were read. Twenty-six full articles were read and 18 were ultimately selected for discussion. In addition, expanded searches and personal collections generated ten further articles that are considered below.

Experimental models

Mouse

Schulman and Kilbourne conducted some fascinating experiments using a murine model in the 1960’s *(123)*. Mice infected with influenza by the aerosol route (Donors) were used to transmit infection to other susceptible mice (Recipients). Transmission was demonstrated between mice housed in the same and separate cages and the frequencies of transmitted infection were similar. One experiment allowed the ventilation in a cage housing Donors and Recipients to be altered; when ventilation was increased, infection rates decreased. These findings were interpreted as signifying that aerosol transmission was dominant.

Ferret

The ferret has been used in the study of influenza since influenza induced rhinitis was first observed *(2)*. The ferret is naturally infected by influenza and demonstrates many similarities to human disease. This has allowed aspects of infection such as the clinical course of infection, pathogenesis and immune response to be studied *(124)*. Ferrets readily transmit infection and they share with humans the same α-2,6 glycosidic linkage to sialic acid receptors in respiratory epithelial cells to which human influenza attaches *(125)*, because of these features ferrets have been regarded as the best animal model *(126)*. However, there are several impracticalities to using ferrets; these include costs (of the animal itself and upkeep) and the need to confirm susceptibility to infection through serologic assays.

In 1941 Andrewes and Glover demonstrated that transmission could occur between ferrets housed in different cages and separated by distances that would arguably only permit aerosol spread. They went on to show that transmission occurred between ferrets connected only by closed ducts (2.5 metres in length some had S or U bends) with airflow moving from Donor to Recipient ferrets, again suggesting the aerosol route was active *(127)*.

Guinea Pig

A human H3N2 virus was shown to replicate well in guinea pigs after intranasal inoculation and transmission from infected to recipient animals occurred when animals were housed together or in separate cages (side by side and separated by 91cm). However, infection did not produce symptoms and no effect on body temperature or weight was seen *(121)*. Palese's group have since used this model to study factors that affect routes of transmission. The relative contributions of droplet/aerosol and fomite (contact) transmission have been studied. Infected and recipient animals were placed in separate cages >80cm above each other and transmissions occurred. However, when recipient animals were placed in the cages of infected animals (infected animals were removed but fomites were not) less infection transmission was seen *(128)*.

Transmission Factors

Host

The period of infectiousness in mice seemed only to occur between 24-48 hours after the initiation of infection, despite the fact virus could be isolated from Donor mice for several days. It was also apparent that some mice transmitted infection more readily than others, in keeping with the theory of super-spreaders (see page 25). Interestingly these super-spreaders did not have significantly different viral titres to others suggesting infectiousness is not solely due to the amount of virus in the URT *(129)*.

One strain of mouse was shown to transmit infection more readily than another strain. The authors postulate that differences in social behaviour could account for this e.g. one strain being more active and aggressive than the other *(122)*.

Immunisation of mice has been shown to affect transmission. Mice immunised with an inactivated virus were more resistant to infection but they were found to transmit infection equally as well as non-immunised mice. In contrast it was found that mice who developed immunity following infection were not only more resistant to subsequent infection but they were less able to transmit it *(130)*.

Environment

The effects of RH and temperature have been studied in animal models. Transmission experiments in mice were noted to be significantly more successful in winter months compared to summer months *(122)* and when RH was manipulated, greater rates of transmission were observed at lower RH *(131)*. Experiments on guinea pigs housed in an environmental chamber were conducted that only allowed for droplet or aerosol transmission. Low RH (20-30%) seemed to favour transmission while higher RH (80%) inhibited it. Possible explanations include; i) an increased stability of influenza virus in aerosols at low RH compared to higher ones and ii) at low RH water will evaporate from respiratory droplets leading to the formation of droplet nuclei . Both explanations favour a role for aerosol transmission in this model. In another set of experiments transmission occurred at low temperature (5^0C) more frequently than higher temperatures (20 and 30^0C). An explanation for the effect of temperature may be that viral shedding was found to be higher and of increased duration at lower temperatures *(70, 132)*.

These findings were further explored by considering contact transmission *(71)*. Recipient guinea pigs were placed in the same cages as infected ones with ambient temperatures of 20 and 30^0C. Transmission was seen to occur equally at both temperatures; the authors suggest that whilst droplet and aerosol transmission is reduced by high temperatures, contact transmission is not (as virus is not released and therefore not exposed to the outside environmental). Based on these findings they go on to suggest that the predominant route of transmission in tropical countries is contact transmission whilst droplet/aerosol transmission is predominant in temperate regions and is subject to seasonal variations (though the validity of this hypothesis has been questioned *(17)*).

Virus

Differences in transmissibility exist both between and within (due to mutations and reassortants) viral sub-types;

- In mice experiments, differences were observed between the ability of different viruses to transmit *(133)*.
- Two groups of mice were infected with different viral strains by aerosol in an experimental chamber that allowed sampling of air. Whilst virus inoculums and virus stability in aerosols were the same, only one of the viral strains could be recovered from aerosols emitted by the infected animals *(133)*.
- In a ferret transmission model the 2009 H1N1 virus was found to transmit equally efficiently as an H1N1 seasonal virus by one group of researchers *(134)* but not by another *(135)*.
- The affects of mutations that confer oseltamivir resistance on transmission have been assessed. In ferrets, viruses with the NA-E119V and the NA-H274Y mutations have been shown to transmit efficiently by contact *(136)*, while an arginine-to-lysine mutation at position 292 abolished transmission *(137)*. In the guinea pig model, contact transmission of an H3N2 virus with the NA-E119V mutation was also efficient, however, aerosol transmission occurred much less frequently *(138)*.
- Avian viruses do not transmit efficiently in ferret models *(139-141)*. To investigate this further researchers have created a variety of avian-human reassortant viruses; some fail to transmit *(139, 142)* whilst others do *(143)*. In a series of experiments using human 1918 – avian H1N1 reassortant viruses it has been shown that the 1918 HA gene allows for contact transmission in a ferret model but that the addition of the 1918 PB2 protein was

necessary to allow droplet/aerosol transmission *(144)*. The importance of the PB2 protein has also been shown by Palese's group *(145)*.

Discussion

Animal studies provide an insight to the multiplicity of factors that seem to have a role in influenza transmission. On a basic level both droplet and aerosol routes appear to play significant roles in transmission in animal models. Unfortunately it is not possible to discriminate between them in most models, though it has been argued that the experimental methods described favour the operation of aerosol over droplet transmission *(8)*.
A better understanding of the viral determinants of transmission is developing *(146)*, though the variety and interplay of traits is complex; some seeming to hinder transmission whilst others permit it through different routes. At present the underlying mechanisms responsible for these differences are not known. It is intriguing to consider that the type vaccine (live v inactivated) given might have an effect on the ability to transmit though no studies have been done in man. Animal work has also allowed us to study environmental factors and the impact they have on transmission routes; temperature and humidity seem to be key in this regard.

Host factors are also important but it becomes increasingly difficult to accept that findings in animals are applicable to humans. Despite the development of valid and reliable animal models it requires a leap of faith to extrapolate animal findings to humans when considering influenza transmission. Disease pathogenesis including immunopatholgy will differ and host factors that contribute to transmission can vary between animal models, for example, symptoms and social and physical behaviours. In humans the existence of super-spreaders appears likely and the possibility that different social behaviours and interactions can affect transmission seems logical. It is difficult to study such human phenomena in animals. Furthermore, animal models do not allow us to test NPIs to reduce transmission such as HH and use of face masks. So whilst animal models are generating useful and important findings their application to humans will always be debatable.

6. Modelling Influenza Transmission

Modelling is an attempt to predict an outcome based on variables (either known or hypothetical) associated with the outcome. Using modelling, a number of authors have tried to estimate the importance of the various routes of influenza transmission with infection resulting from a particular route being the outcome. The development of a plausible model, however, is not always straight forward because a large number of parameters need to be taken into account. Furthermore whilst some of the parameters have been well characterised e.g. dynamics of aerosols many others have not which undermines the reliability of a model.

Search Methods

The authors own reference collection and bibliographic searches of selected articles were used to identify papers where modelling has been used to investigate the modes of influenza transmission. In addition, PubMed searches looking for related citations to those already selected were performed.

Studies

Atkinson & Wein (39):
A model constructed on an infinite set of differential equations was developed to quantify the roles played by the aerosol and contact routes of influenza transmission. It was based on a household containing four individuals, one of whom is infected and includes an array of some 40 parameters (covering viral dynamics, route of infection and infectious doses) which have been estimated from available literature (Figure 9).

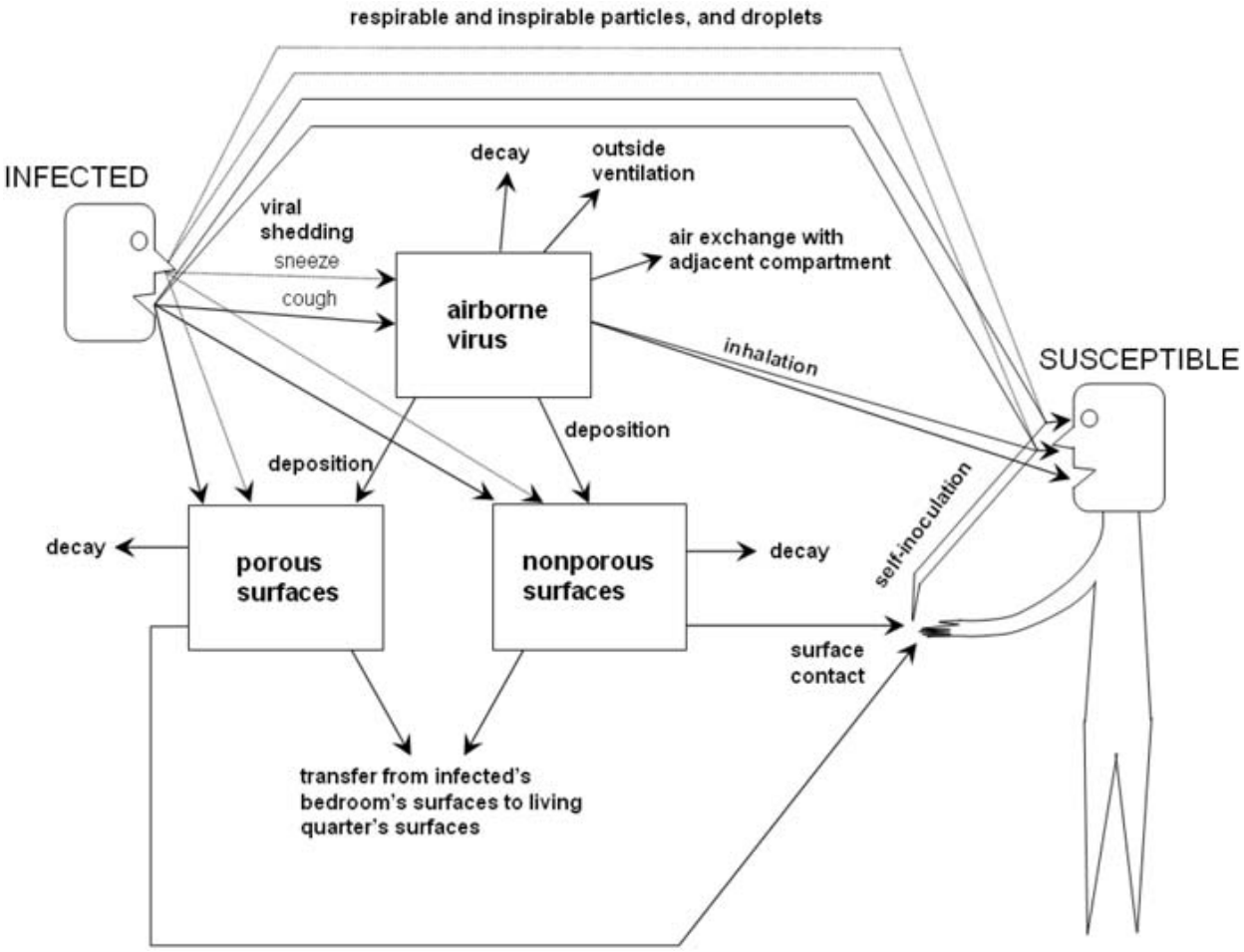

Figure 9; reproduced from *(39)*: A graphical depiction of the model.

They find that; i) most transmissions occur early in an infected person's illness, in fact over half occur in the pre-symptomatic period; ii) a caregiver is twice as likely to be infected than a non-caregiver; iii) a very small proportion of virus exits on small aerosol particles; iv) virus survives in air longer than it does on hands and v) the infectious dose for virus in aerosols is much smaller than that in either droplets or settled particles. The model leads them to conclude that aerosol transmission is far more dominant than contact transmission.

In the above model, the environment into which a virus is expelled (air and surfaces) plays a significant role. The authors also consider the situation where virus interaction with the environment is much less – the case of droplet transmission whereby droplets travel quickly and directly from infected to susceptible persons. This can only occur during certain close expiratory events; an estimate of this is made by considering the infectious potential of an

isolated close range (60cm) cough or sneeze. It is assumed that shedding is in the order of 10^7 TCID50 with 99% of this being emitted in a sneeze and 1% in a cough. They find that a 'perfect' (directed precisely so that particles land on mucous membranes) cough or sneeze carries an infection probability of 0.011 and 0.981 respectively, though it must be stated that a 'perfect' cough or sneeze may be a rare event.

Nicas & Best (35):
The hand to face contact route of transmission was investigated by Nicas and Best. A study was performed which assessed hand to face touching behaviour in a group of ten volunteers and data was then used in an algebraic model to estimate the dose of influenza that could be transmitted in this way. The average contact rate was 15.7 per hour. Other parameters included; viral titres deposited on surfaces, area of surfaces touched, transfer rate of virus between surface and hand, viral loss from surfaces and hands, and infectious dose. The scenario was a caregiver attending a sick family member in a bedroom for 30 minutes. An infection risk due to hand contact of 0.011% is generated. The authors note uncertainty regarding the infectivity parameters used but by using perhaps better estimates than the authors did, Tellier still finds the modified risk of 1% to be rather low *(8)*.

Nicas & Jones (147):
Recognising that routes of infection are not mutually exclusive, Nicas and Jones consider the relative contribution of four influenza exposure pathways; a) contaminated hand to facial membranes contact, b) inhalation of respirable particles that can reach the distal lung (aerosol), c) inhalation of particles that do not penetrate further than the tracheobronchial region (droplet) and d) droplet spray onto facial membranes. Using an exposure pathway model (Markov chain model) and the setting of a coughing, bed-ridden patient, they estimate the relative contribution made to infection risk by the four routes described when a visitor enters the patient's room for 15 minutes. For a number of parameters (virus emission rates, virus inactivation rates, viral loads, virus transfer rates, respirable particle inhalation and hand exposure, droplet spray exposure and inspirable particle exposure) they have used published data to estimate values. They highlight two important variables:

1) Infectious dose - Because there is considerable uncertainty about dose-response data they use two scenarios. The first uses an infective dose ratio LRT:URT of 3200:1 and the second a 1:1 ratio

2) Viral titres - A range of viral concentrations in saliva are used in the model

When the infectious dose ratio is 3200:1 routes a), b) and d) all contribute substantially to infection risk; 27%, 14% and 58% at a virus saliva concentration of 10^4ml and 58%, 31% and 11% at a concentration of 10^8ml respectively. When the ratio is 1:1 a) is the most important with c) and d) playing lesser roles; 93%, 3.3% and 3.7% at viral concentration of 10^6ml respectively.

The authors note that the above figures are based on the assumption that all virus delivered to mucous membranes will reach target receptors; this however is unlikely to be the case. As a result the authors add a variable (ε) to the model - the fraction of virus deposited on membranes that reaches target cells. The effect of this is much greater on routes a) and d) such that if ε = 0.01 and the LRT:URT ratio is 3200:1 respirable particles contribute over 90% to infection risk.

Stilianakis & Drossinos (148):
Stilianakis and Drossinos constructed an epidemiological model incorporating the dynamics of inhalable respiratory droplets to assess their importance in infection transmission. Three influenza epidemics are modelled, each mediated by a different route of transmission. The model is based on the susceptible-infective-recovered epidemic model with inhalable droplets acting as agents of transmission. Key, are the dynamic physical properties of emitted respiratory droplets which are covered in over 20 model parameters.

Epidemics mediated by:

- Respirable droplets (<10μm) – the model creates an epidemic peaking at day 78 and lasting for 150 days. It reproduces the characteristic transmission dynamics of influenza with a basic reproduction number R_0 of 1.28. Small respirable droplets are the dominant mode of spread followed by larger and then settled respirable droplets

- Inhalable droplets (10-100μm) – a faster epidemic peaking at day 28 with an R_0 of 2.31 is simulated, the authors assert that such a scenario may present in a closed population such as a school or nursing home where high attack rates are seen. Close contact between individuals in these situations is necessary for inhalable droplets to act as they settle rapidly in air.
- Settled droplets – if the hand to face contact time is 20 seconds the epidemic peaks at day 23 with an R_0 of 2.57, again typical of outbreaks in closed settings where these highly infectious particles (compared to respirable particles) are brought into play.

These findings do seem to concur with observations of influenza outbreaks and, as the authors point out, suggest that epidemic duration is inversely proportional to the viral load of a particle.

Spicknall et al (149):
Again in this paper four modes of influenza transmission are considered (definitions as outlined above). The authors in this case constructed a discrete event, continuous time, stochastic transmission model and sought to analyse the factors that affect the transmission from one person to another. Transmission parameters (22 in total) were selected after considering an epidemiological triad for environmentally mediated transmission; it consists of i) Host – susceptibility, contagiousness and behaviour, ii) Agent – contagiousness, transferability, survivability, infectivity and iii) Environment – surface area to volume ratio, type of host present, transferability, survivability and host density. Parameter values are obtained from published data as well as 'expert judgement'. An abstract venue is modelled; it is of fixed volume, contains a virus emitting (coughing) person and is visited by susceptible persons. To simulate the model, 18 parameters are constrained within reasoned limits and 10,000 parameter sets are generated. A basic reproductive number is produced specific to the mode of transmission operating.

The indirect contact mode of transmission has the highest R_0 (1.7) followed by droplet (contact) (0.27), respiratory (0.05) and inhalatory (droplet) (0.006) routes, however, the authors explain that indirect contact transmission is not necessarily dominant in all settings. For example, parameter sets exist that give dominance (R_0>1.7) to each route in isolation of the others; out of 10,000 sets indirect contact was dominant in 3079, respiratory in 121 and droplet (contact)in 66. The inhalatory route was never dominant alone. Furthermore, considerable

overlap is also seen where modes appear co-dominant, this occurred in 1969 sets. The authors state that features of the host, pathogen and environment all play a role in determining transmission mode dominance.

Further analyses were then performed to identify specific parameters that determine transmission intensity. High and low intensity contact transmissions are principally determined by the infectious dose delivered to the URT (URT ID_{50}), the self inoculation rate and the shedding magnitude (amount of virus present in the environment). Droplet transmission is differentiated by the URT ID_{50}, host density and shedding magnitude whilst respiratory transmission is differentiated by host density, proportion of virus that is respirable, shedding magnitude, LRT ID_{50} and lung deposition fraction.

Discussion

Modelling offers a fascinating and important insight into the multifarious processes that affect the different routes of influenza transmission. Studies attempt to combine defined physical dynamics with biologic processes to reveal outcomes. Whilst the models and input data that feed them have important differences, most support the concept that all transmission routes can be important given the right circumstances, though the droplet route appears least significant - whilst these particles are high in number and have high infectivity potential their inability to reach target cells is highlighted in the models.

Most authors conclude that transmission likely occurs through multiple routes and that it is dependent on the value of parameters acting at a given point in time. Nicas and Jones state that if NPI are to be used they must account for all exposure routes but Spicknall et al go further to suggest that we may be able to predict which modes operate in specific scenarios by taking account of key factors. This in turn might allow the most effective interventions to be employed.

There are however significant limitations to each of these models:

- The empiric data that they rely on is weak. Many crucial variables arise from studies undertaken many years ago and both the reliability and validity of data is questionable

- The assumptions and data that some models have used is often open to debate *(8)*
- The models are restricted to certain scenarios, e.g. a coughing patient being visited in a bedroom. They cannot possibly take account of the huge variety of other factors; patients being mobile rather than bed-ridden, particle emission through talking, breathing and sneezing as opposed to coughing alone, heterogeneity in particle emission (e.g. super-spreaders) and room ventilation changes through door and window opening

Nevertheless important determinants of infection risk have been highlighted; viral shedding of patients, infectivity of influenza at different sites, host density and virus transfer efficiencies. By focusing future research on these areas and obtaining better data, models can be improved and they will become invaluable in helping us to appreciate the roles played by the different routes of transmission.

Evidence from Modelling

- Modelling studies offer insight into variables that combine to influence whether a particular route of transmission can occur
- Models constructed to date seem to suggest roles for both the contact and aerosol routes; droplet transmission is given less prominence
- However, models are wholly dependent on the parameters input (assumptions); unfortunately a lack of robust data in many areas (e.g. human infectious dose) and difficulties in capturing the dynamic process of transmission (as opposed to a defined event) limit their contribution

Conclusion

The evidence that informs the debate on the routes of influenza transmission comes from many sources and is varied in what it reveals. A weakness of this evidence is that investigating routes of transmission was seldom the primary aim of the studies reviewed and this may explain some inconsistencies. However, having reviewed the available data a more likely explanation is that all routes of transmission can have a role to play and that their relative significance will depend on a set of circumstances acting at a certain time. Dictating the process are factors related to the virus itself, the host and the environment. These findings are summarised in Table 4.

There is sound physical evidence that influenza virus can exist and survive on droplets and aerosols both in the air and on fomites, such that transmission via these particles could occur from the respiratory tract of an infected individual to target cells of a susceptible contact. On the basis of available evidence the indirect contact route of transmission appears to be most vulnerable to natural interruption. In order for infection to be transmitted; a) titres of virus high in excess of the infectious dose must be shed, b) deposited virus must survive, c) high titres must be collected via hands, d) virus must survive on hands, e) hands must deposit an infectious dose of virus on target cells. However, some biological evidence gaps pertinent to all routes remain; i) the infectious doses for each route are uncertain and ii) a lack of evidence to demonstrate the presence of infectious and transmissible virus in natural settings. Until these gaps can be addressed by further studies that employ novel methods and technology, the relative importance of each of the proposed routes will remain uncertain.

In the outbreak investigations reviewed, no routes of transmission can be completely excluded. Long range transmission is rarely reported though it is difficult to distinguish which route of infection has occurred after close ‘contact’ with an infected individual; all are possible. The circumstances described in several of the reports do seem to support the aerosol route. This is important as it provides evidence to suggest that given the opportunity, not only is the aerosol route of transmission possible, it may also be significant. Furthermore, two of the three studies in which aerosol transmission appeared significant were located in hospital environments.

If interventions can reduce transmission through a particular route (e.g. HH) or different routes (SFM vs. respirators) then useful data may emerge. It is difficult to ignore the HH study conducted by Talaat et al. which suggests a prominent role for the hands in the transmission of influenza. Face masks also seem to reduce the transmission of influenza; SFM act on two potential routes of transmission whilst respirators act on all three. To date respirators seem no more efficacious than SFM in preventing transmission suggesting that the aerosol route is not significant though several other factors could be influencing this e.g. filtering ability of SFMs (large variation exists – data reviewed elsewhere), quality of fit of respirators (not all studies have used fit tests), inadequate power of studies and use of end points that are not influenza specific.

Human challenge studies suggests that a smaller viral dose is needed to initiate infection in the lower compared to the URT. Studies using antivirals administered via different routes also suggest that the LRT is the preferred site of infection. These data are important as they corroborate our expectations of biological plausibility, i.e. that lower doses of virus in aerosols are required, compared to doses in droplets and on fingers. However, the ability of experimentally induced infection to act as a surrogate for natural infection is often questioned. The droplet and aerosol routes of transmission dominate in transmission experiments with animals but we should caution against dismissing the contact route as minor; experimental methodologies may bias against it and the markedly different social and physical behaviours of humans compared to small mammals are probably critical. Nevertheless, the fact that sound evidence for the existence of aerosol transmission is available is notable. There also seems little doubt that some environmental factors e.g. temperature and humidity can affect transmission; we may be able to deploy such knowledge in infection control strategies.

Modelling offers a fascinating insight into the variables that combine to influence whether a particular route of transmission can occur. Models constructed to date seem to suggest roles for both the contact and aerosol routes; droplet transmission is given less prominence. However, transmission is a process that can be affected by a large number of parameters and to get valid outcome from a model, parameters need to be known and well defined. Unfortunately a lack of robust data, particularly concerning factors such as infectious doses and viral shedding, and the difficulty in capturing the dynamic process of transmission (as opposed to a defined event) limit their ability to tell the full story.

Evidence	Indirect Contact	Droplet		Aerosol
		Direct contact	Inhalation	
Plausibility	Virus can survive on fomites and hands but transmission must overcome several hurdles to occur and because of this the process appears tenuous.Few data are available to show viable virus exists on hands or fomites in natural conditions	Virus is present and can survive on droplets. The bigger the droplet the higher the titre of virus that can be present. This is important as the URT requires a high infectious dose. However, the chance of a droplet reaching its target cell is low	Virus is present and can survive on droplets. The bigger the droplet the higher the titre of virus that can be present. This is important as the URT requires a high infectious dose. However, it would require a perfectly directed cough or sneeze to enable this route. Larger droplets cannot penetrate the LRT	Virus is present and can survive on aerosols. Low titres of virus may be present but this may be compensated by the ability to penetrate the LRT where lower infectious doses are required. Few data are available to show viable virus exists on aerosols in natural conditions
Outbreak investigations	'Close contact' identified as important but difficult to distinguish between routes as all can act at short range	'Close contact' identified as important but difficult to distinguish between routes as all can act at short range		'Close contact' identified as important but difficult to distinguish between routes as all can act at short range. However, circumstances in three studies appear to support the existence of the aerosol route
Interventions	The HH study by Talaat et al provides convincing data that this route of transmission is significant	SFMs show some effectiveness at reducing transmission but we are unable to say whether droplet, indirect contact or both are interrupted		Respirators appear no more effective than SFMs at reducing transmission suggesting a minor role for aerosol
Challenge studies	Virus has been recovered from fomites around experimentally infected volunteers but no studies have been done specifically assessing contact transmission	Infection can be initiated following direct nasal inoculation. The URT appears to need a higher infectious dose than the LRT. Nasally applied zanamivir does not prevent infection		Infection can be initiated by inhaling/respiring aerosols. The URT appears to need a higher infectious dose than the LRT. Inhaled zanamivir prevents experimental (intranasal) infection but not natural infection.
Animal studies	Does not appear significant but cannot infer that this is the case in humans	'Close contact' identified as important but difficult to distinguish between routes as all can act		Convincingly shown to be active in several studies
Modelling	Support for this route is apparent but relies heavily on assumptions	Support for this route is limited based on assumptions made that reaching target cells is problematic		Support for this route is apparent but relies heavily on assumptions

Table 4: Summary of available evidence for the proposed routes of transmission

If the setting in which transmission occurs is important in determining the route of transmission, we should consider some features that might be present in two common scenarios; hospital and homes.

Hospital:

- Patients requiring hospital treatment are likely to be more unwell and this could be associated with more symptoms and higher and /or prolonged viral shedding with an increased likelihood of transmission by all routes. Patients in hospital are likely to be relatively more confined than those in the community which will allow higher concentrations of virus to build up as aerosols and on surfaces. Confined settings are a feature of many outbreaks
- A high number of susceptible individuals may come into contact with a hospitalised patient; e.g. HCWs, other patients, visitors, other hospital staff. HCW vaccination (if available) is clearly important.
- Aerosol generating procedures are largely confined to hospital; e.g. chest physiotherapy, assisted ventilation (non-invasive and invasive)
- Engineering controls are more likely to be in place in hospitals; e.g. negative pressure ventilation rooms which will reduce aerosol loads. However, this is a limited resource and many patients are nursed outside of these areas. Natural ventilation, for example via windows, is useful but is often not possible in hospital rooms

Homes:

- Peak viral shedding occurs early in the course of infection when individuals are perhaps more likely to be in their own homes increasing the likelihood of transmission by all routes
- Caregivers (family / friends) are often not vaccinated and do not usually have the availability of personal protective equipment e.g. face masks which could protect against droplet transmission

Further research into routes of transmission is needed and has been called for by many authorities *(3-5, 150)*. Focus should be placed on i) studies with the primary aim of investigating modes of transmission, ii) the key determinants of transmission identified by modelling, e.g. infectious dose and iii) intervention studies ranging from face masks to

engineering controls. Careful consideration of the evidence presented in this review could highlight specific risks in given situations and lead to infection control plans that mitigate them.

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