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Predicting "Airborne" Influenza Viruses: (Trans-) mission Impossible?

E.M. Sorrell[a], **E.J.A. Schrauwen**[a], **M. Linster**[a], **M. De Graaf**[b], **S. Herfst**[a], and **R.A.M. Fouchier**[a,*]

[a]National Influenza Center and Department of Virology, Erasmus Medical Center, Rotterdam, The Netherlands, P.O. Box 2040, 3000CA Rotterdam, The Netherlands [b]Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, United Kingdom

Abstract

Repeated transmission of animal influenza viruses to humans has prompted investigation of the viral, host, and environmental factors responsible for transmission via aerosols or respiratory droplets. How do we determine – out of thousands of influenza virus isolates collected in animal surveillance studies each year – which viruses have the potential to become "airborne", and hence pose a pandemic threat? Here, using knowledge from pandemic, zoonotic and epidemic viruses, we postulate that the minimal requirements for efficient transmission of an animal influenza virus between humans are: efficient virus attachment to (upper) respiratory tissues, replication to high titers in these tissues, and release and aerosolization of single virus particles. Investigating "airborne" transmission of influenza viruses is key to understand – and predict – influenza pandemics.

Introduction

The virus or virus subtype that will cause the next influenza pandemic is a highly debated topic in the field. Some believe that only influenza virus subtypes H1, H2, and H3 can cause pandemics in humans, and therefore – beyond isolated cases of zoonotic infections – we should not worry about virus subtypes such as H5N1, H7N7 or H9N2 for human health. Many believe that swine viruses, rather than avian viruses, are more likely to cause the next pandemic. However, beyond the fact that there will be future pandemics, there is little known in terms of the viral origin, subtype, and virulence of the next pandemic. One other assumption can be made: the virus will be transmissible via small particle aerosols (typically <5μm) or large respiratory droplets (typically >5μm), shortened hereafter as airborne transmissible.

Influenza A viruses are constantly undergoing genetic and phenotypic changes during their circulation in avian and mammalian species. Our knowledge of viral traits necessary for host switching and virulence has increased significantly over the last decade. However, what exactly determines airborne transmission of influenza viruses in humans has remained largely unknown. Only when we fully understand the viral (genetic and phenotypic), host, and environmental factors that drive airborne transmission can we start to make predictions about which influenza viruses may cause future influenza pandemics.

Corresponding Author Ron A.M. Fouchier, Erasmus Medical Center, P.O. Box 2040, 3000CA Rotterdam, The Netherlands, Phone +31 10 7044066, Fax +31 10 7044760.

Past pandemics

Four major pandemics have been recognized for which viral genome sequence data is available. While it was initially proposed that the 1918 H1N1 Spanish influenza pandemic was caused by a wholly avian virus that adapted to humans [1], recent evidence suggests that some of its genes were derived from mammalian viruses circulating as early as 1911 [2*]. The 1957 H2N2 Asian influenza pandemic resulted from the reassortment of avian HA, NA, and PB1 virus genes with the then circulating seasonal human H1N1 influenza virus [3]. The H3N2 Hong Kong influenza pandemic of 1968 was also a product of reassortment between avian and human virus genes; HA and PB1 genes of the H2N2 virus were replaced by those of an avian H3 virus [3]. In 2009, an H1N1 influenza virus of swine origin caused the first pandemic of the 21st century [4]. The gene constellation of this virus showed clear evidence of multiple reassortment events that had presumably occurred in pigs over a period of years [5] (Figure 1). The role of swine as a mixing vessel for the generation of reassortant influenza A viruses with pandemic potential is generally accepted, yet still underestimated (reviewed in [6]). However, it should be noted that reassortment can conceivably take place in avian or human hosts; for pandemics prior to 2009, there is no evidence that reassortment events occurred in pigs. Regardless of the identity of the mixing vessel it is important to emphasize that most, if not all, recent influenza pandemics were caused by reassortant viruses. The expansion in surveillance efforts in pigs in response to the 2009 pandemic will most likely reveal many more reassortant viruses that may or may not have the potential to infect and spread in humans. But how, out of thousands of animal influenza viruses from surveillance studies, can we select the ones we should prepare for as potential causes of new pandemics?

Viral determinants of transmission

Retrospective analysis of pandemic H1 (1918), H2 and H3 viruses has revealed that only one to two mutations in the HA receptor binding site are required to confer binding preference for virus receptors on cells of the upper respiratory tract (URT) of humans, α2,6-linked sialic acids (SAs) [7]. Partially borrowing from this knowledge, several mutations in the HA protein, including Q222L, G224S, E186D, K189R, S223N and N182K (H5 numbering), have been shown to change and/or increase receptor binding of avian H9N2 and H5N1 viruses to human URT tissues [8-12] with changes like Q226L (H3 numbering) improving replication and transmission of H9 viruses [13]. However, to date, none of the “designer” H5N1 viruses carrying these mutations have resulted in airborne transmission [11,14]. Efficient H5 transmission may thus require more subtle differences in receptor preference than simple α2,3 (the receptor for avian viruses) versus α2,6 SA linkage specificity.

Previous research has also pointed to key changes in the polymerase proteins that increase virus replication efficiency at 33°C, the accepted temperature for efficient replication in the mammalian URT [15*-16]. These changes at positions 627, 701 and 591 of PB2 have also been shown to support transmission of multiple subtypes in mammalian models [17-19*]. A decrease in association of the PB2 and NP proteins in mammalian cells is thought to be the mechanism behind the increase in replication efficiency [20].

PA and NP genes have also been associated with viral host restriction but the key amino acids have yet to be identified. Even fewer experiments have looked at the roles of NA, M and NS proteins in determining host range and transmission. It is likely that virus tropism, efficiency in replication, amount of virus shed, and the duration of shedding are important factors for transmission efficiency. A longer duration of virus shedding at high titer may be

hypothesized to increase the chance for the virus to reach susceptible host(s) and therefore increase transmission events [21**].

Airborne transmission; size does matter

Human-to-human transmission of influenza viruses can occur through contact, direct or indirect, and/or respiratory droplets (large droplets and aerosols). Opinions differ on the importance of each mode of transmission (reviewed in [22-23]). The role of each has been well studied in mammalian models, focusing on the ferret and guinea pig (reviewed in [24*] and Table 1). Efficient aerosolization of viral particles is however crucial for a virus with transmission efficiency and pandemic potential. There is no exact particle size cut-off at which transmission changes from exclusively large droplet to aerosol. However it is generally accepted that for infectious particles with a diameter of 5μm or less, transmission occurs through aerosols. Large aerosol droplets do not remain suspended in air and typically travel < 1m before settling on the mucosa of close contacts or environmental surfaces. In contrast, smaller particles, < 5μm, have a slow settling velocity and can thus travel further than large droplets [25]. Humans exhale droplets of widely varying size and quantity [26] and the generation of aerosol particles by coughing or sneezing has been well documented, with the majority of particles expelled during breathing and sneezing measured at < 1μm [27-28]. Evidence to support the role of aerosols in influenza transmission include the prolonged persistence of infectivity in aerosolized influenza at low humidity [29-30], transmission to volunteers by aerosols reproducing the disease at doses much less than required by intranasal infections [31] and the abolishment of transmission when virus aerosolization is blocked with UV treatment of upper room air [22].

H1N1, the next pandemic

The continuing spread of highly pathogenic avian influenza (HPAI) H5N1 viruses in poultry and the consistent, albeit infrequent, transmission to humans with high mortality rates [32-33] has kept H5N1 a top candidate on the list of potential future pandemics. It has been suggested that human-to-human transmission between family members in close contact has occurred [34-36] however, sustained human-to-human transmission has not been confirmed. It is this lack of human-to-human transmission that has prevented extensive infection, and therefore prevented an H5N1 pandemic.

The inefficient airborne transmission of HPAI H5N1 virus has been confirmed in several mammalian models including ferrets, mice and guinea pigs (Table 1). Numerous studies with wild type H5N1 viruses, reassortants between H5N1 and human viruses, H5N1 viruses adapted by repeated passage, and “designer” H5N1 viruses with mutations known to increase virus binding and/or replication have failed to yield airborne H5 viruses, showing direct contact transmission at best [14,17,37-39]. This highlights the complexity of the mechanism(s) of influenza virus transmissibility and confirms that H5N1 viruses require further adaptation to become a pandemic threat.

Along with H5N1, H9N2 viruses have become enzootic in poultry in large parts of Eurasia [40]. These H9N2 viruses increasingly display human-like receptor specificity [10,13] and have occasionally transmitted to humans and pigs [41-42] with most human cases likely going unreported due to the relatively mild symptoms associated with infection. In the laboratory, H9N2 viruses have been shown to transmit to direct contact ferrets with no prior adaptation or mutations [13] and compared to H5N1 virus, were easily adapted after reassortment in a human H3N2 backbone to become airborne in the ferret model [43]. Recent work indicates that reassortment of an H9N2 virus, within the backbone of the pandemic 2009 H1N1 virus, supports aerosol transmission in the ferret without any further adaptation [44*]. Therefore, currently circulating avian H9N2 viruses are able to create a

potentially pandemic virus when provided the opportunity for reassortment with a human-adapted virus.

Virus design; why and how do influenza viruses become airborne?

The major challenges for influenza virus transmission research going forward are the types of studies needed to elucidate mechanisms for transmission. In our opinion, the focus should be on “gain of function” approaches rather than “loss of function”. For the purpose of virus transmission studies, loss of function experiments are like destroying a car engine; remove any crucial part and the engine will stop running. In analogy, mutating a transmissible virus so it no longer transmits is a pointless exercise, giving us none to little mechanistic information; there are a thousand ways to accomplish that. Gain of function experiments mimic tuning the car’s engine; only one or a few parts need tuning but the key is determining which part(s) out of the possible thousand they are. To investigate which viral parts need “a tune up” before it becomes transmissible, at least two options are available, and both should be followed. First, we could “replay” the evolutionary events leading to the pandemic viruses of e.g. 1957 and 1968. Which genetic changes in the avian-origin viral genes made these reassortant viruses airborne? Such experiments are now ongoing in several laboratories. Secondly, we can hypothesize, based on accumulated data, which viral characteristics would facilitate airborne transmission. Below, we discuss several of these features that we believe are important for influenza to become airborne. We postulate that the minimal requirements for airborne influenza viruses are 1) Attachment to and replication in appropriate cells of the URT; 2) High virus yields in the URT; 3) Virus shedding as single particles.

Attachment and Replication

Viruses with binding preference for α2,3 SAs can infect and replicate in human lungs and can lead to severe clinical symptoms and even death, yet these viruses are limited in their ability to infect the URT and subsequently transmit via aerosols (Figure 2A). Thus far, all transmissible viruses bind to α2,6 SAs and are capable of attaching to, replicating in, and transmitting from the URT (Figure 2B). However, the gross receptor binding profile alone does not guarantee transmission. Therefore, efficient transmission may require more subtle differences in receptor preference than α2,3 or α2,6 SA linkage only. Glycan arrays and other assays for virus attachment may facilitate investigation of these subtle differences in binding specificity, but identification of the critical influenza virus receptors on the cells of the URT and LRT is needed to facilitate future research on host and tissue specificity as well as transmission.

High virus yields in the URT

When the extent of virus replication of airborne viruses is low for example, after vaccination with seasonal live attenuated viruses, transmission generally does not occur [45*-46] (Fig 2C). To date, all pandemic viruses have established efficient and productive infections in the URT (Fig 2D). The mechanism necessary for URT tropism has been linked to affinity for α2,6-SAs and adaptive mutations supporting replication at ~33°C, which optimizes molecular interactions between viral proteins and cellular host factors [16-19]. High levels of virus replication in the URT may ensure that large amounts of progeny virus are released into aerosols from the nose and mouth upon sneezing, coughing, or breathing.

Virus shedding as single particles

Low NA activity can result in inefficient cleavage of SAs and as a consequence, inefficient release and aggregation of virus particles resulting in little to no successive rounds of replication (Figure 2E). The substrate specificity and activity of NA and HA must be in

some balance with respect to receptor binding and cleavage in order to maximize the yield of progeny shedding as single virus particles that are efficiently transmitted (Figure 2F). In addition to HA, the NA's role in virus transmission warrants further investigation.

Unknowns

There are many unknowns when it comes to airborne transmission. The above factors are only a starting point for research. More work is needed to elucidate the relative contribution of human influenza virus transmission via contact, (large) respiratory droplets, and aerosols. With respect to airborne routes, how much does sneezing and coughing add to transmission in comparison to breathing alone? How representative are the available animal models (guinea pigs, ferrets) for transmission in humans? Guinea pigs generally do not sneeze upon influenza virus infection yet transmit viruses via the airborne route and some viruses are not transmitted between ferrets despite frequent sneezing of the animals [24]. Neither of these results provides definitive proof for a role of coughing and sneezing in transmission. Other important questions are whether airborne viruses come from one or many cell types, whether virus shape is important for transmission or virus stability and whether key changes that drive transmission of one viral subtype are applicable to every other viral subtype. Research and attention should be focused on answering these questions regarding the mechanisms involved in airborne transmission. It is crucial to sustain the current funding, energy and collaboration in order to answer these key questions, which may in the future, be able to inform approaches to prioritizing risk from other emerging viruses.

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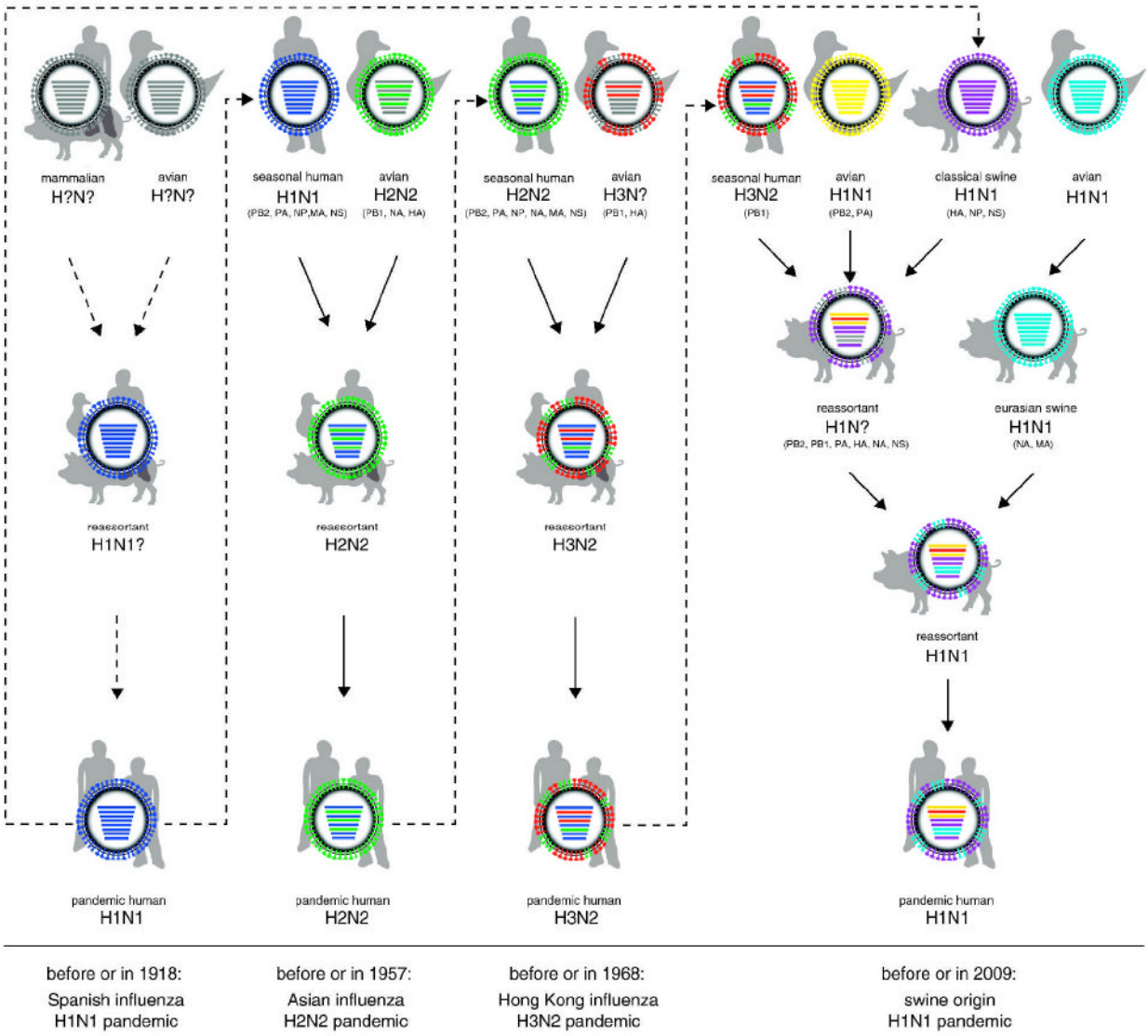

Figure 1. Reassortment and adaptation events of pandemic influenza A viruses

For the 1918 H1N1 “Spanish influenza” pandemic, evidence for two mutually exclusive scenarios has been presented: the gradual adaptation of avian genes to the human host and a reassortment event between avian and mammalian viruses. After 1918, the H1N1 virus caused seasonal epidemics until 1957, when the H2N2 influenza A virus emerged upon reassortment between the seasonal H1N1 and an avian H2N2 virus, introducing the avian HA, NA, and PB1 genes. This H2N2 virus circulated in humans until 1968, when reassortment of the H2N2 with an avian H3 virus resulted in exchange of the H3 HA and PB1 genes to yield a new pandemic virus of subtype H3N2. The 2009 H1N1 pandemic contained the NA and M genes of the Eurasian swine lineage, and the other genes of a “triple reassortant” swine influenza virus that earlier acquired its genes upon reassortment between human, avian, and (classical) swine viruses. Grey colour in virus particles indicates uncertainty of viral gene segment origin or lack of data. Dotted arrows indicate uncertain scenarios and solid arrows indicate events that are supported by scientific evidence. Dashed arrows represent pandemic viruses circulating in following influenza seasons.

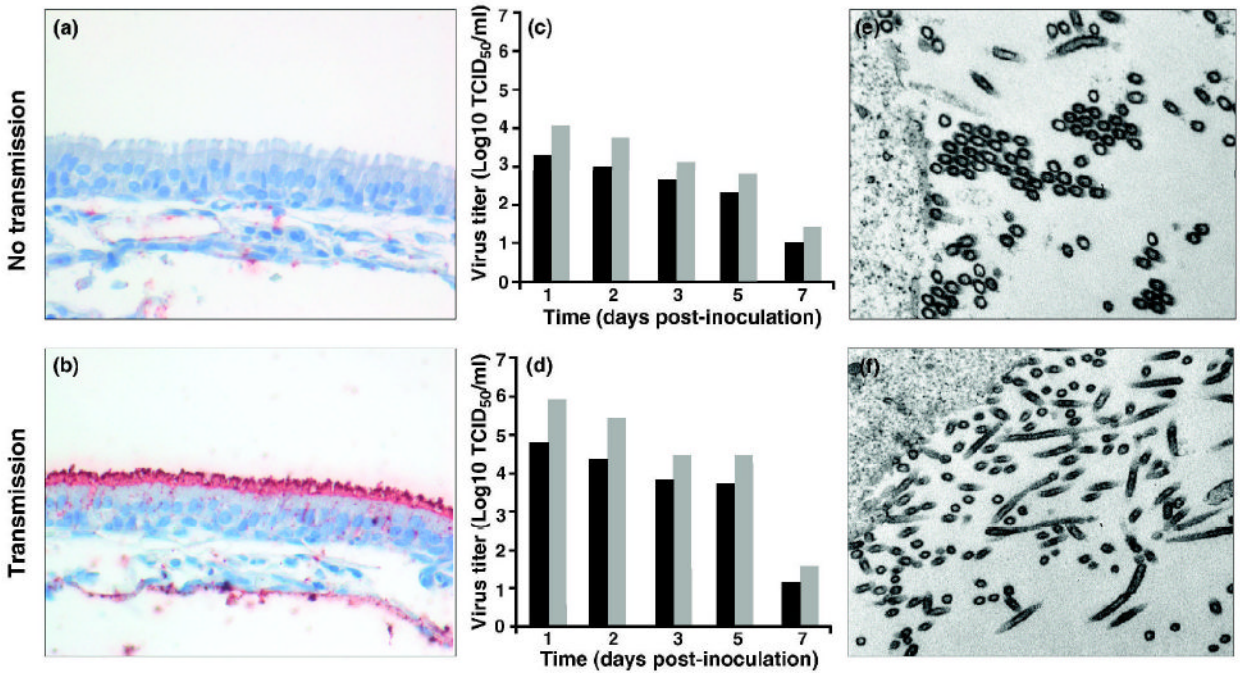

Figure 2. Proposed minimal requirements for influenza viruses to become airborne

Figures represent theoretical virus subtypes with non-transmissible (virus 1) and transmissible (virus 2) phenotypes, unpublished data. Virus attachment to ferret URT is visualized with FITC-labelled viruses. (A) Shows no attachment of virus 1 to URT whereas (B) shows red staining indicative for attachment of virus 2 to the ferret URT. Virus yield in the URT of ferrets (intranasal infection) is shown for virus 1 (C) and virus 2 (D), with grey bars representing virus shedding from nose swabs and black bars from throat swabs. Electron microscopy of viruses budding from human 293T cells. (E) Shows viral aggregates released in clusters of spherical particles of virus 1 while (F) shows single virus 2 particles being released from the infected cell as both spherical and filamentous virions.

Table 1

Mammalian Models for Influenza Transmission

Model/Species	Virus Subtype		Transmission[a]	Reference
Mouse (MF-1/CFW)[1]	H1N1	Seasonal	A[1]	[47-50]
	H2N2	Seasonal	A[1]	[49]
		Pandemic	A[1]	[49]
Mouse (Balb/C)	H1N1	Seasonal	None	[38]
		Pandemic	None	[38]
	H3N2	Pandemic	None	[38]
	H5N1	HPAI	None	[38]
Ferret	H1N1	Seasonal	A	[51-52]
		Pandemic	A	[53-56]
	H3N2	Seasonal	A	[13-14,57]
		Pandemic		
	H1/H3	Oseltamivir resistant	D/None	[58-60]
	H2N2	Pandemic	D/A	[61]
	H5N1	HPAI	D[2]/None	[11,14,37]
	H7N7	HPAI	D	[62]
	H7N2		D/None	[62]
	H7N3		D/None	[62-63]
	H9N2		D/A	[13,42]
Guinea Pigs	H1N1	Seasonal	D[2]/A[2]	[38,64-65]
		Pandemic	D/A	[66]
	H3N2	Seasonal	D/A	[38,64-65]
	H1/H3	Oseltamivir resistant	D	[67]
	H5N1	HPAI	D[2]/None	[15,66]
	H1	Swine	A[2]/None	[17,65]
	H3N2	Swine	None	[65]
	H9N2		None	[65]
Hamsters	H1N1	Seasonal	D[2]	[68]
	H3N2	Seasonal	D	[68]

[a] A = airborne transmission, D = direct contact transmission, None = no transmission direct or airborne

[1] Transmission may have been due to differences in mouse strain and/or husbandry techniques (bacterial co-infections were likely to play a role in the 1960s studies)

[2] Partial transmission found