Influenza A virus reservoirs and intermediate hosts: dogs, horses, and new possibilities for human infections

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Summary

Influenza A virus (IAV) infections in hosts outside the main aquatic bird reservoirs occur periodically. Although most such cross-species transmission events experience limited onward transmission in the new host, sustained influenza outbreaks have occurred in poultry and in a number of mammalian species including humans, pigs, horses, seals and mink. Recently, two distinct strains of IAV have emerged in domestic dogs, with each circulating widely for several years. Here, we briefly outline what is known about the role of intermediate hosts in influenza emergence, summarize our knowledge of the new canine influenza viruses (CIVs) and how they provide key new information on the process of host adaptation, and assess the risk these viruses pose to human populations.

Pathways to emergence of human influenza viruses. Avian influenza viruses frequently spillover from their reservoirs in aquatic birds and infect humans, but are generally poorly transmissible (1-3). It is often thought that the infection and transmission of avian influenza viruses among non-human mammals, particularly swine, facilitates their subsequent transfer to humans in a form that can be readily transmitted (2, 4-7), although evidence for such "intermediate" mammalian hosts in the emergence of human influenza viruses is limited. Among the few clearly documented examples is the 2009 H1N1pandemic virus, where there is strong evidence that it emerged in humans following the transfer of a swine influenza virus, itself comprised of gene segments of swine, human, and avian origin (8, 9). In addition, human viruses can frequently ‘spill-back’ to pigs (10, 11), suggesting that they comprise a single virus gene pool. Indeed, anthropozoonotic infections are far more common in pigs than are human infections with swine influenza viruses (12). The emergence of the H1N1 influenza virus in 1918 involved parallel epidemics of the virus in humans and swine, although it is unclear whether the virus emerged in humans and then spread to swine, or the reverse (13). Similarly, although re-assortment between avian and human viruses resulted in the emergence of the H2N2 (with new
HA, NA, and PB1 gene segments) and H3N2 (with new HA and PB1 gene segments) pandemic viruses in 1957 and 1968, respectively, conclusive evidence of involvement of swine or another mammalian intermediate is lacking.

Currently only a small number of mammalian hosts are recognized as sustaining IAV transmission (Figure 1), and it is not clear what distinguishes these species from those for which influenza has not been reported. Influenza viruses appear to be relatively host specific in mammals. A variety of barriers to virus infection are known, including innate immune responses, sialic acid receptors, as well as a number of intracellular factors, and some viral mutations that overcome these barriers to facilitate replication and spread in new hosts have also been identified (14). Establishment of IAVs in new hosts is also dependent on the structure of the host population, which must be sufficiently large and interconnected to allow the sustained spread of the virus. In the last few centuries the human population has increased significantly in size and connectivity, as have those of domestic food animals such as poultry, swine and cattle in intensive agriculture, companion animals (cats and dogs) in households or in kennels or shelters, and some small animals raised in large numbers for fur or meat (15-17). Bats may also be present in large, interconnected populations, and appear to maintain their own IAVs, although these are phylogenetically distinct and have not so-far been detected in other mammals, such that they are not an important reservoir (18, 19).

The emergence of canine influenza viruses. The inventory of mammalian hosts that are known to sustain transmission of IAVs has increased in the past 15 years due to the emergence of two strains of the virus in dogs: the H3N8 equine influenza virus (EIV) that transferred to dogs in the USA in about 1999, and the avian-like H3N2 virus that transferred to dogs in Asia around 2005 or 2006. Both these CIVs have continuously circulated in the dog population since they emerged, creating many opportunities for exposure in humans and other species.
Equine and canine H3N8 influenza viruses. Horses have been natural hosts for sustained influenza transmission on at least three occasions, with an outbreak of an unknown equine influenza virus (EIV) subtype occurring in 1872 (20), an H7N7 EIV that emerged around 1956 (21), and an H3N8 EIV that was recognized around 1963 when there was an outbreak of respiratory disease in Florida occurring soon after the importation of horses from Argentina (22). The H3N8 EIV appears to have emerged from an avian influenza virus that spread to horses, and in the 1960s and 1970s there was some re-assortment of that virus with the H7N7 viruses that eventually died out during the 1970s (23). Limited transmission of H3N8 EIV to dogs has been observed on a number of independent occasions, including in the UK (2002) (24) and Australia (2007) (25). However, despite these spillover events, only one EIV transfer that occurred around 1999 in the USA resulted in the establishment of H3N8 canine influenza virus in dogs in Florida, which involved the transmission of a single EIV from horses. This outbreak was first described in 2004 as the cause of disease among racing greyhounds, and within a year the virus was transferred to racing tracks in many parts of the USA (26), and subsequently to other dog populations and breeds where it has been maintained ever since (27, 28). Notably, since about 2008 H3N8 CIV appears to have been primarily maintained in animal shelters in a few large cities where dogs live in dense populations and where susceptible animals are introduced at high rates (27, 29, 30). Because infected dogs are frequently transferred from these shelters, the virus is often passed to other dog populations where it causes outbreaks, but where it is seemingly unable to sustain long-term transmission, likely because of overly heterogeneous contact networks (27, 29). In particular, although there are an estimated 80 million susceptible household dogs in the US, they appear to lack the degree of contact necessary to continuously transmit influenza virus such that chains of transmissions quickly die out (29). As a result, CIV infections in small shelters or in the household dog populations die out within days or weeks (27, 29). To date, no re-assortment has been detected by the H3N8 CIV with any other human or animal influenza viruses.
It is therefore clear that horses acted as an intermediate host for the emergence of the canine H3N8, although only after about 37 years of continuous circulation of the virus, suggesting that there are major adaptive or epidemiological barriers to CIV emergence. Since its emergence, the H3N8 CIV lineage has diverged from the EIVs that have continued to circulate in horses, with distinct amino acid substitutions in each gene segment, some of which may have been selected for canine adaptation (30, 31). For example, certain amino acid substitutions in the sialic acid binding site modify the replication of the virus in the respiratory tract of the dog by altering the binding to the sulfated glycans, and likely other changes to sialic acid binding (32). Those EIV strains that circulated at the time of emergence of CIV appear to readily infect dogs after experimental challenge or by housing dogs with EIV infected horses (33, 34), and can infect canine tracheal explant cultures (35), so that the amount of adaptation required to infect dogs with these viruses is likely low. Notably, there is currently no evidence for transfer of a CIV back to horses in nature, and CIV replicates inefficiently when used to challenge horses or horse tracheal cells (36-38). Besides the infection in dogs, the H3N8 influenza has also been reported to infect pigs in China (39), and to infect cats in experimental studies (40).

**H3N2 canine influenza virus.** In contrast of H3N8, the H3N2 CIV most likely arose around 2005 or 2006 in China but was first recognized in South Korea around the same time, and the virus or specific antibodies have since been detected in many parts of China and Korea, as well as Thailand (41-44). The epidemiology of the H3N2 CIV is still poorly understood, but some reports indicate moderate seroprevalence in some household dog populations, and it has been transferred both over long distances within and between the three countries in which has been detected (45). A role for populations of farmed dogs may aid in the maintenance of the virus (46). H3N2 CIV is an avian-like virus and all isolates share a single common ancestor, but the circumstances of the transfer are unknown, and its gene segments are related to both Eurasian
and American avian influenza lineages (42, 47). H3N2 CIV appears to have a relatively broad host range, and the virus naturally infects cats and spreads among them under some circumstances (48). Under experimental conditions, H3N2 CIV has also been shown to infect ferrets, albeit with limited transmission (49, 50).

Despite its recent emergence, reassortants between H3N2 CIV and other influenza viruses have been described, including a CIV carrying the M segment of human influenza virus H1N1 (51). Another CIV has been suggested to harbor the NP segment of an avian influenza virus related to H5N1 viruses (46), although that is not a clear reassortant (P. Murcia, unpublished results). Notably, an H3N1 influenza virus carrying the H3 of CIV and the other gene segments from the H1N1 pandemic human influenza virus has also been reported (52). This should be cause of concern as previous influenza pandemics have originated when viruses harboring a novel HA are introduced into the human population.

Intermediate hosts and CIV spread to other species (including humans)? There are only a small number of well-documented examples of mammalian intermediate hosts allowing virus transfer onward to new hosts, or acting as “mixing vessels” facilitating reassortment leading to new outbreaks. In addition the human H1N1 pandemic virus, and the likely role of humans in the emergence of the classical H1N1 virus in swine in 1918, horses clearly played a role in the emergence of the H3N8 virus in dogs. The CIV/human virus reassortant reported for the H3N2 CIV means that we should be alert to the possible emergence of intact or reassortant CIVs in humans. Such a threat results from the extensive and sustained contacts between humans and their pet dogs, which would result in significant human exposure to CIV if the viruses spread widely among household dogs. It is also possible that additional re-assortments between canine and human viruses could occur in either dogs, humans, or possibly other hosts such as cats, potentially creating a new human influenza virus for which there may be little or no natural immunity. Clearly, these risks may vary depending on the virus, with H3N2 CIV seemingly...
posing a greater risk that H3N8 CIV (see below). Importantly, to date there have been no documented transmissions of H3N8 EIV or CIV to humans despite the close interactions between humans and horses since the first appearance of EIV in the early 1960s, or dogs since 2000 (53, 54). However, direct experimental inoculation of human volunteers with EIV in the 1960s showed that infection did occur, but only to low levels (55). These results suggest that there are high intrinsic barriers to the establishment of H3N8 in humans, such that onward transmission in two mammalian hosts (horses and dogs) does not guarantee successful infection of another (humans).

In contrast, the observation of H3N2 CIV:human reassortants in dogs shows that co-infection of one of the hosts (likely dogs) is possible and hence a cause for concern. In addition, H3N8 EIV has been isolated from pigs in Asia (39) and from bactrian camels in Mongolia (56), and swine are susceptible to infection with many avian-like and human viruses (10, 11). However, the ability of canine influenza viruses to infect pigs (and vice versa) has not been formally tested.

Control strategies and CIV eradication. Despite the emergence and spread of the CIVs in both the USA and Asia, there may still be opportunities to control or even eradicate the viruses while they are in their reservoir hosts. Although the household dog population in the USA is about a quarter the size of the human population, the transmission of influenza among dogs appears to be much less efficient than for influenza viruses among humans (27, 29). After 14 years of continuous dog-to-dog transmission, H3N8 CIV is still only circulating in a sustained fashion among dogs in a small number of large animal shelters, and the transmission rate is sufficiently low that the virus may be eradicated from those shelters by reducing the rate of transmission, or reducing the efficiency of infection (29). The spread of the H3N2 CIV in Asia may likewise also be controlled by timely interventions, particularly if the household dog
population is similarly inefficient in allowing virus transmission, so that the virus is being maintained in other dog rearing or housing facilities.

### Concluding remarks

The emergence of canine influenza viruses, like the emergence of any influenza lineage in a novel mammalian host population, may constitute a direct or indirect pandemic risk. However, adaptation to mammals should not be seen as a direct conduit to emergence in humans. Clearly, there are important host barriers that have yet to be understood or breached. While the lack of transmission of EIV or CIV to humans suggests that long-standing infection of a mammal does not necessarily indicate a threat to humans, a better understanding of the ecological, evolutionary and molecular mechanisms of influenza emergence is essential to accurately determine which viruses pose a risk to human health.

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REFERENCES


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<td>49</td>
<td>Lee YN, Lee DH, Park JK, Yuk SS, Kwon JH, Nahm SS, Lee JB, Park SY, Choi IS, Song CS.</td>
<td>2013</td>
<td>Experimental infection and natural contact exposure of ferrets with canine influenza virus (H3N2).</td>
<td>The Journal of general virology 94:</td>
<td>293-297</td>
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FIGURES

Figure 1. Schematic showing the known emergence events of influenza A viruses in mammals that lead to extended outbreaks including epidemics and pandemics. The diagram indicates the known mammalian hosts (humans, swine, seals, mink, horses, and dogs), as well the approximate period during which those were known to sustain natural transmission in that host. The viruses are all assumed to have emerged from avian reservoirs to infect the initial mammalian hosts. The three known transfers between different mammalian hosts that went on to establish epidemics in the new hosts are indicated by red lines, and the first mammalian host therefore acted as the intermediate reservoir host for the transferred virus. The direction of transfer of the 1918 H1N1 virus is not known for certain, but is now thought to have transferred from humans to swine, rather than the other way around. The transfers of new AlV gene segments into the human virus lineage are shown by the vertical arrows and the segments listed; these are assumed to involve a direct transfer of the gene segments from the avian ancestor in humans. There are complex mixtures and transfers of genome segments among some of the viruses in swine, but most of those are not shown because they do not involve transfer between different hosts.