Review

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Reverse zoonosis of influenza to swine: new perspectives on the human–animal interface

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The origins of the 2009 influenza A (H1N1) pandemic in swine are unknown, highlighting gaps in our understanding of influenza A virus (IAV) ecology and evolution. We review how recently strengthened influenza virus surveillance in pigs has revealed that influenza virus transmission from humans to swine is far more frequent than swine-to-human zoonosis, and is central in seeding swine globally with new viral diversity. The scale of global human-to-swine transmission represents the largest 'reverse zoonosis' of a pathogen documented to date. Overcoming the bias towards perceiving swine as sources of human viruses, rather than recipients, is key to understanding how the bidirectional nature of the human–animal interface produces influenza threats to both hosts.

Swine as reservoirs for IAV diversity and pandemic threats

IAVs are considered to be one of the greatest threats for the next global pandemic given the abundance of permanent animal reservoirs harboring viruses that occasionally spill over into humans [1]. For decades, swine have been thought to serve as intermediate 'mixing-vessel' hosts (see Glossary) for the evolution of pandemic viruses because of their capacity to be infected with both avian and human influenza viruses, which can exchange genome segments via a process termed 'reassortment' to generate new pandemic viruses [2–4]. This threat was borne out in 2009 when the first influenza pandemic of the 21st century was caused by a novel reassortant swine H1N1 virus with genetic segments from both avian-like Eurasian swine viruses and North American swine viruses [5]. Since 2009, the genetic diversity of IAVs in swine populations (swIAVs) has expanded globally, generating additional pandemic threats such as the novel variant H3N2v swIAV that infected more than 330 humans in the United States during 2011–2013 [6]. Determining the evolutionary mechanisms that increase IAV diversity in swine is therefore important for human and animal health.

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The evolutionary dynamics of IAVs are determined by their complex multi-host ecology, viral structure, and segmented genome. IAVs are single-stranded (ss), negative-sense RNA viruses of the family *Orthomyxoviridae* that infect a wide range of avian and mammalian species. The genome of IAV (total length ~13 kb) is composed of eight ssRNA segments that encode at least 12 viral proteins. Two surface glycoproteins, the hemagglutinin (HA) and neuraminidase (NA), are the main viral antigens and are used to describe the diversity of IAV subtypes (e.g., H3N2). Sixteen antigenically distinct HA and nine NA subtypes exist in wild aquatic birds, which are considered to be the reservoir hosts for IAV diversity [7]. Avian influenza viruses occasionally spill over into humans (e.g., H5N1 and H7N9), usually with little onward transmission. On rare occasions, major influenza pandemics occur when an avian influenza virus evolves the capacity to transmit human-to-human, as occurred in 1918, 1957, and 1968 [8,9]. Reassortment was key in generating the novel avian–human reassortant H2N2 and H3N2 viruses associated with the pandemics of 1957 and 1968 [9],

Glossary

Antigenic drift: the continual evasion of host immunity by the gradual accumulation of mutations in the hemagglutinin (HA) and neuraminidase (NA) surface glycoproteins that change the antigenic structure of the influenza A virus (IAV).
Bayesian phylogeography: the inference of viral migration patterns using time-scaled phylogenies inferred in a Bayesian framework in which prior knowledge assesses the probability of model parameters in the presence of new data.
Hemagglutinin (HA): an influenza virus surface glycoprotein, which binds to sialic acid receptors to assist viral entry into host epithelial cells. Eighteen HA serotypes are present in animal species.
Mixing vessel: a host species with the capacity to be coinfected with multiple genetically distinct viruses of different animal origins that are able to reassort to generate genetically novel viruses. Swine are considered to be 'mixing vessels' for reassortment between human and avian influenza viruses.
Molecular clock: the observation that the rate of molecular evolution is relatively constant for a given lineage, which provides a useful tool for estimating the timing of events in evolutionary history.
Pandemic: an influenza outbreak associated with a new viral subtype that occurs over a large geographic area (often globally).
Reassortment: a form of genetic recombination in which two influenza viruses coinfect a single cell and exchange entire RNA segments to form genetically novel viruses.
Reverse zoonosis: an infectious disease that can be transmitted from a human to a non-human host (sometimes by a vector). Also sometimes referred to as 'anthroponosis'.
Zoonosis: an infectious disease that can be transmitted from a non-human host to a human (sometimes by a vector).

respectively, although the proposed role of swine as intermediary mixing-vessel hosts remains unclear. Avian influenza viruses also have evolved to be transmissible in non-human mammalian hosts: avian-origin H3N8 viruses have transmitted in horses since the 1960s [10], avian-origin H3N2 has circulated in dogs in Asia since the mid 2000s [11], and avian-origin H1N1 has circulated in Eurasian swine since the 1970s [12]. IAVs also can jump between mammalian species, as evidenced by the adaptation of equine H3N8 viruses to canines in the early 2000s [13] and swine H1N1 viruses to humans in 2009 [5]. One determinant of host specificity is the distribution of sialic acid receptors that bind the viral HA glycoproteins on host cells to facilitate viral entry [14]. Understanding what molecular evolutionary changes allow particular IAVs to jump between host species, and the potential role of swine as intermediary hosts, remain central questions in influenza virus research (Box 1).

In the aftermath of the 2009 H1N1 pandemic there has been great interest in understanding the threat of swIAVs for humans, including (i) estimates of the frequency of human infection with swIAVs [15,16], (ii) assessments of the pandemic potential of various swIAVs in animal models [17,18], and (iii) increased surveillance of global swIAV diversity in regions and continents that rarely or never previously reported swIAVs, including Africa [19], Latin America [20–23], South Asia [24,25], and Australia [26,27]. Paradoxically, the swIAV sequence data collected to assess the pandemic risk of swine for humans have demonstrated that humans transmit far more IAV diversity to swine than pigs have ever transmitted to humans, at least in terms of viruses that transmit onward in the new host (as opposed to dead-end infections). In this review we describe the central role of human-to-swine transmission events in the evolution of IAV diversity in swine. In particular, since 2009 the continual introduction of pandemic H1N1 (pH1N1) viruses from humans has expanded the genetic diversity of swIAVs globally, introducing new challenges for the control of influenza in swine, pandemic threats for humans, and important research questions related to the human–swine interface.

Humans as major sources of influenza virus diversity in swine

While there is historical evidence for influenza outbreaks in humans, poultry, horses, and even canines dating back several centuries [28], there is evidence of only one possible localized influenza virus outbreak in swine in England before the 1918 H1N1 'Spanish flu' pandemic [29]. During the early stages of the 1918 pandemic, outbreaks of the virus were identified in both humans and swine in the USA [30], and it remains impossible to determine whether humans first transmitted the virus to pigs, or vice versa, owing to the lack of data from that time [31]. From 1918 to 1998 this 'classical' swine H1N1 influenza virus, isolated in swine in 1930 [32], exhibited relatively limited antigenic evolution and caused low concern for swine producers. Beginning in the 1970s, the global evolution of swIAVs became much more complex with the introduction of an avian H1N1 virus and a human seasonal H3N2 'Port Chalmers-like' virus into European swine herds, and subsequent reassortment events between them (a detailed historical characterization of global influenza virus diversity in swine has been provided in several recent reviews [33–35]).

Central to the global evolution of genetic diversity of swIAVs is the repeated introduction of IAVs of human seasonal virus origin in swine [21,36–41]. In contrast to major pandemic influenza events that last 1–3 years and occur sporadically, seasonal influenza viruses are associated with annual epidemics in humans that occur in winter in temperate regions. It remains difficult to estimate the full extent of human-to-swine transmission of seasonal viruses historically, owing to large gaps in swIAV surveillance geographically and in past decades (Figure 1). However, a conservative global estimate of 20 discrete human-to-swine transmission events was recently inferred by phylogenetic analysis of whole-genome viral sequence data [42] (Figure 2). No clear patterns of geographical distribution or any 'hot zone' of human-to-swine transmission of IAVs are apparent once sampling bias is removed. Instead, these events have occurred multiple times on all four continents that conduct at least minimal swine surveillance: North America, South America, Asia, and

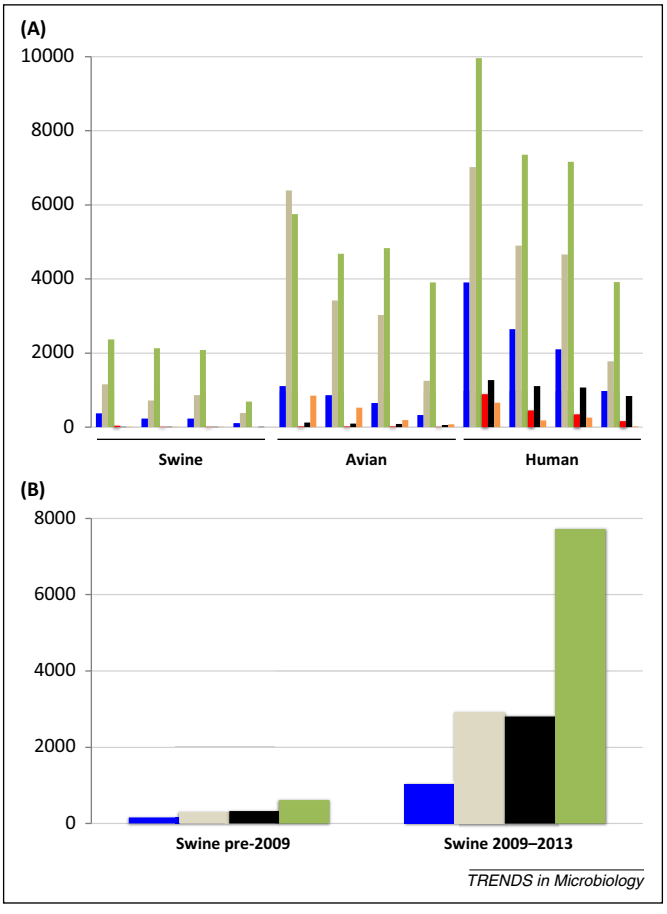

Figure 1. Influenza A virus (IAV) sequence data available for swine; birds; and humans. **(A)** Numbers of IAV sequences available at the Influenza Virus Resource [120] at GenBank for swine, birds, and humans for the full-length hemagglutinin (HA) segment; full-length neuraminidase (NA) segment, whole genome, and the main antigenic region of the HA (HA1), by region. The bars representing the number of viral sequences from Europe are shaded blue; Asia, light brown; North America, green; South America, red; Pacific region, black; and Africa, orange. **(B)** Numbers of swine influenza virus sequences available at GenBank for the full-length HA segment, full-length NA segment, whole genome, and partial HA gene, for the time periods 1931–2008 and 2009–2013.