Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

and colleagues report that simply delaying a third dose until vaccine antibody levels wane can alleviate B cell suppression. RTS,S trials are already examining delayed doses for their effects on antibody responses and efficacy (Regules et al., 2016). The report in this issue of *Cell Host & Microbe* suggests RTS,S developers are on the right track.

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Recipe for Zoonosis: How Influenza Virus Leaps into Human Circulation

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The features that permit or prevent a virus from becoming a zoonotic threat is an ongoing area of investigation. In this issue of *Cell Host & Microbe*, Herfst et al. and Henritzi et al. help define the molecular and host determinants of influenza virus spillover from animal to human populations.

Humans are continually threatened by the ongoing emergence and circulation of potentially zoonotic viruses in wild and domestic animal populations. Understanding which, if any, of these viruses pose a threat to human health requires understanding of characteristics of the virus, the host, and the species-specific barriers that must be overcome. In this issue of *Cell Host & Microbe*, two separate groups report key findings that extend our understanding of the molecular and host determinants that could drive influenza virus spillover from animal to human populations (Figure 1).

Zoonotic transmission and establishment of a novel virus in the human population are largely constrained by three features: the opportunity to spill over from the animal host, the capacity to transmit and replicate in the human population, and the ability to escape human immunity. First, spillover from the reservoir or intermediate host must occur through direct or indirect contact between an infected vector and the naive host. Second, the virus must be able to transmit effectively and replicate in a human host. This is constrained by receptor-mediated entry to cells and the replication competence in that new host environment. Third, to successfully establish itself in the human population, successful zoonotic viruses must escape human immunity, including innate

defenses and antigenic memory-recall responses.

Herfst et al. (2020) bring attention to the second constraint: the ability of an influenza virus to infect and transmit between mammals. Their focus is on avian-origin A/H10N7 influenza viruses associated with the 2014 outbreak in European seals with mortality estimates of up to 10% (van den Brand et al., 2016). Previous studies on molecular determinants underlying mammalian versus avian infectivity have zeroed in on receptor specificity as a major constraint to cross-species transmission. The hemagglutinin (HA) of influenza virus binds to sialic acids that decorate glycoproteins on the cell surface, either in the α2,3 avian-specific or α2,6 mammalian-specific linkages. In the seal-adapted, avian-origin A/H10N7 virus, sequence analysis revealed that minor variants with mammalian markers in the HA receptor binding site were present within the viral population. Of great interest to this work were two mutations in HA, Q226L and G228S, that have been previously suggested as determinants of zoonotic potential in avian influenza viruses (Tzarum et al., 2017). The team found that seal viruses containing the Q226L HA mutation predictive of human-like binding showed increased transmissibility between ferrets in comparison with the parental virus. Upon transmission, the Q226L mutation was positively selected for in the respiratory contacts, suggesting its potentially advantageous action during mammalian infection.

Aside from changes to human-like binding, transmission between mammals also requires HA-acid and temperature stability. Once bound to the surface receptor, the influenza virion must be internalized and mediate an escape from the endosome to replicate. This action requires a stable HA protein that mediates membrane fusion upon conformation change resulting from acidification of the endosome. Previous work in swine-origin influenza viruses has shown that reductions in the pH of fusion are a hallmark of human adaptation. In 2009, the H1N1pdm virus became increasingly acid stable in human transmission, as evident by the activation pH of HA dropping from highs of up to 6.0 in swine precursor viruses to less than 5.5 in later human isolates (Russier et al., 2016). Paralleling these findings, the presence of the Q226L variant plus two other stabilizing mutations in HA of seal H10 viruses were sufficient to drop the pH of fusion from greater than 5.7 to 5.2 in the putative avian precursor virus, suggesting the virus potentially has pandemic potential in humans.

In summary, the studies by Herfst et al. (2020) demonstrate there is not one "silver bullet" change required for an avian-origin virus to cross into human circulation. More than one molecular determinant, specifically the HA Q226L, T244I, and E74D mutations, collectively enable human-like receptor binding at high avidity and HA stability. Importantly, this work also highlights the role that seals play in adapting avian-origin viruses to a new mammalian host and potentially acting as an intermediary to its spillover into humans (Karlsson et al., 2014).

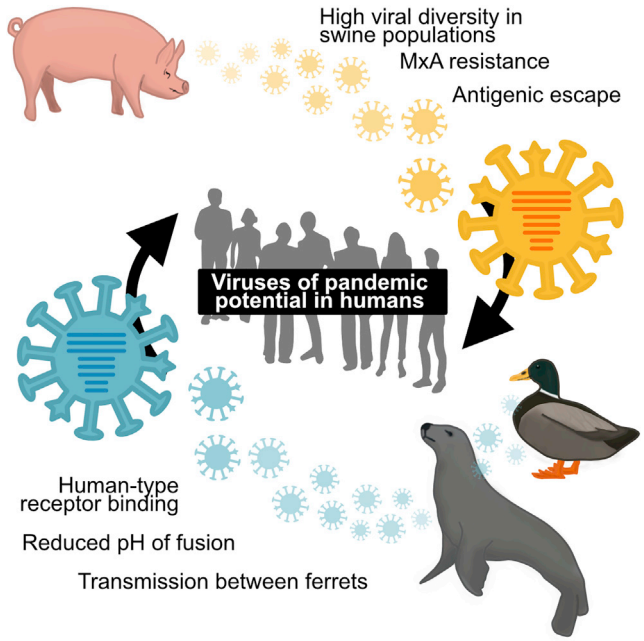

Figure 1. A Recipe for Zoonosis
Delineating the molecular and host restrictions that permit or prohibit viral spillover from wild, agricultural, and domestic animal species is crucial in understanding the true risk the vast number of viruses that circulate globally pose to humanity. Here, Henrizi et al. (2020) and Herfst et al. (2020) discuss the characteristics in swine and avian-origin viruses that potentially encode their pandemic potential.

Henritzi et al. (2020) focus on the third ingredient for zoonoses to occur: escape of human immunity. Here, the focus turns to swine: a common source of novel influenza viruses with proven pandemic potential. Given the importance of swine in cross-over infections into people, there are robust surveillance programs maintained across the world. This group takes advantage of a passive-surveillance program yielding over 18,000 distinct samples to characterize the viruses circulating in European swine. The initial genomic analyses identified four phylogenetically distinct swine virus lineages, novel reassortments among those lineages and several "reverse zoonotic" introductions of H1N1pdm origin internal gene segments, which we know from the 2009 pandemic can replicate in humans.

Because these novel swine influenza viruses have molecular signatures suggesting their ability to replicate in a human host, the group sought to determine their propensity to jump the next hurdle in establishing widespread transmission in humans: escape of immunity. Upon infection, the immune response begins with physical and non-specific innate defenses to quell the invading pathogen, including the induction of interferon signaling and the production of interferon stimulated genes (ISGs). However, influenza can "shut down" this host interferon response and escape the attack. Most of this is attributed to the non-structural 1 protein, yet other gene segments can harbor specific mutations rendering them resistant to specific ISGs, including the potent MxA protein. Analysis of the swine viruses identified three distinct clusters, including a fully resistant phenotype harboring the three MxA escape mutations defined in H1N1pdm virus, partially resistant, and weakly resistant strains

(Dornfeld et al., 2019). Only viruses with MxA resistant markers transmitted to contact ferrets, which showed signs of infection and successfully seroconverted. In addition, the European swine viruses are resistant to human memory-recall responses. Many of the swine reassortant viruses showed little cross-reactivity with existing neutralizing antibodies from banked human sera and had significant antigenic distance as measured by antigenic cartography. Together, this suggests the presence of novel swine-origin viruses that can replicate efficiently in mammalian hosts with the ability to escape both innate and memory responses.

The ongoing SARS-CoV-2 pandemic is a stark reminder that a zoonotic virus can spill over anytime from any source, highlighting the need for global and sustained active and passive surveillance programs in animal reservoirs, spillover species, and at the animal-human interface. These programs, highlighted by the cohorts utilized within these two studies, provide a unique opportunity not only to understand the circulating viruses but also, more importantly, to accurately assess the risks they might or might not pose to human and animal health. The works within this issue describe the pandemic potential of currently circulating viruses in mammalian species, focusing on known and novel molecular determinants of binding to human cells, capacity to replicate within this new host, and ability to escape antigenic memory. The culmination of these findings suggests that the breadth of viruses circulating in domestic swine, as well as avian-origin viruses that have adapted to a mammalian host, can in particular harbor genetic signatures, including HA-receptor specificity and stability, escape of human innate immunity, and evasion of human antigenic immune memory, together reading as a recipe for pandemic potential.

Unfortunately, the next flu pandemic is not a matter of if but when. Where the spillover will occur and whether it will be from a bird or mammal is unknown. These studies highlight that influenza viruses from wild birds and swine can gain the molecular determinants needed to successfully infect, replicate, and transmit in mammals including humans. These studies provide important new molecular and host determinants important for spillover infection, which will be invaluable in ongoing and future characterization of emerging influenza viruses.

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