

The virus is out of the barn: the emergence of HPAI as a pathogen of avian and mammalian wildlife around the globe

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ABSTRACT

Highly pathogenic avian influenza (HPAI) has persisted as a One Health threat whose current circulation and impact are addressed in the companion Currents in One Health by Puryear and Runstadler, *JAVMA*, May 2024. Highly pathogenic avian influenza emerged as a by-product of agricultural practices and adapted to endemic circulation in wild bird species. Over more than 20 years, continued evolution in a complex ecology involving multiple hosts has produced a lineage that expanded globally over the last 2 years. Understanding the continued evolution and movement of HPAI relies on understanding how the virus is infecting different hosts in different contexts. This includes understanding the environmental factors and the natural ecology of viral transmission that impact host exposure and ultimately evolutionary trajectories. Particularly with the rapid host expansion, increased spillover to mammalian hosts, and novel clinical phenotypes in infected hosts, despite progress in understanding the impact of specific mutations to HPAI viruses that are associated with spillover potential, the threat to public health is poorly understood. Active research is focusing on new approaches to understanding the relationship of viral genotype to phenotype and the implementation of research and surveillance pipelines to make sense of the enormous potential for diverse HPAI viruses to emerge from wild reservoirs amid global circulation.

Keywords: highly pathogenic avian influenza, One Health, avian influenza virus, HPAI, LPAI

Introduction

Highly pathogenic avian influenza virus (HPAIv) has spread into all continents via movement in both domestic poultry and transmission and movement through wild avian species.¹ Evolving lineages in the last few years have produced a significant expansion of both the geography and species in which the virus has been recovered, including spillover into an expanding array of mammalian hosts in different environments.²⁻⁶ Since its emergence in the late 1990s,⁷ the Guangdong H5 HPAIv lineage has been perceived as a threat to both the global poultry industry and to human health, largely because of immune naivete to the subtype and the clinical phenotype of viral infection. Advances in understanding both the ecology and biology of the virus have provided insight into its origins and pathology of infection, but the threat remains to animal and human health in large part because much remains unknown about the

parameters governing the course of viral evolution and the details of how molecular changes in the virus impact viral phenotype, including the ability to infect and emerge as a pathogen in a novel host. H5 and H7 HPAI have been a continual threat to agricultural poultry as a result of the spontaneous emergence of novel strains of these 2 subtypes in high-density poultry operations in a highly pathogenic form that causes the rapid death of infected birds.⁸

LPAI and the Origins of HPAI

Wild waterfowl as a reservoir for low pathogenicity avian influenza (LPAI) virus was identified in the early 1970s following its discovery in terns (seabirds in the family Laridae, which includes gulls) in South Africa of an H5N3 influenza virus lineage distinct from those identified in poultry outbreaks.⁹ Initially, scientists looking for the virus in wildlife hosts were misled by the initial identification in terns, believing that the virus, as in humans, primarily caused a respiratory disease. When researchers began to look at cloacal swabs from migratory waterfowl, they were able to amplify the influenza virus in culture from a wide variety of avian hosts. Subsequent research has

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shown how common the carriage of influenza virus is primarily in migratory waterfowl but in a wide variety of avian hosts worldwide.¹⁰ The LPAI viruses are highly diverse, driven both by the high mutation rate of influenza as an RNA virus and by the ability of influenza to reassort its 8 single-stranded negative-sense RNA genome segments. Sixteen hemagglutinin (HA) subtypes and 9 NA subtypes have been identified to circulate in wild bird populations and have served as a reservoir for the spillover of the virus into other hosts including mammals, where endemic circulation can sometimes be established. This includes canine and equine hosts where these viruses are a significant disease concern.^{11,12} Low pathogenicity avian influenza (LPAI) is frequently a problem for domestic poultry operations,¹³ and where H5 and H7 viruses are the cause of infection, the transformation to HPAIv has taken place via the insertion of a highly basic cleavage site in the HA molecule that allows the viral HA to be cleaved and activated by systemic proteases and thus allows the virus to infect tissues systemically. In the late 1990s, one of these viruses moved back into wild bird populations in China causing mortality in many species and spread of HPAIv. This H5 viral lineage has evolved over more than 20 years and became established as a circulating strain of HPAI in wild birds. In late 2022 and early 2023 strains of this lineage, which had moved out of Asia and caused outbreaks in Europe and Africa, moved into North America and began a rapid spread throughout the Americas and globally.⁷

Disease Ecology and Comparative Biology

What is clear is that few would claim to have predicted the more than 20-year evolution of this H5 lineage in wild bird species and the dynamics of its recent spread. Research in natural populations has revealed important broad strokes about how the virus amplifies in young, naïve hosts, transmits across flyways, mixes at wintering grounds in the northern hemisphere, and reassorts and diversifies in reservoir species. However, the field has relatively sparse knowledge to help predict the predominance and dynamics of viruses in practical models. Additional effort is needed both to understand viral dynamics in natural environments of both hemispheres and across the major continents and flyways and to understand the way that a variety of hosts, both avian and other, may impact those dynamics, through the amplification, reassortment, and transmission of virus over time and space.

The 2 HPAI clades that currently predominate circulation are clade 2.3.2.1 and clade 2.3.4.4. (Figure 1).^{14,15} In the Americas, several strains of 2.3.4.4 were introduced with one branch that has spread beyond the northeast and dominated circulation. For circulation of the LPAI virus, a normal pattern may see introduction through migratory waterfowl in the fall and southward migration months, followed by reassortment, diversification, and transmission to other species in winter feeding areas or

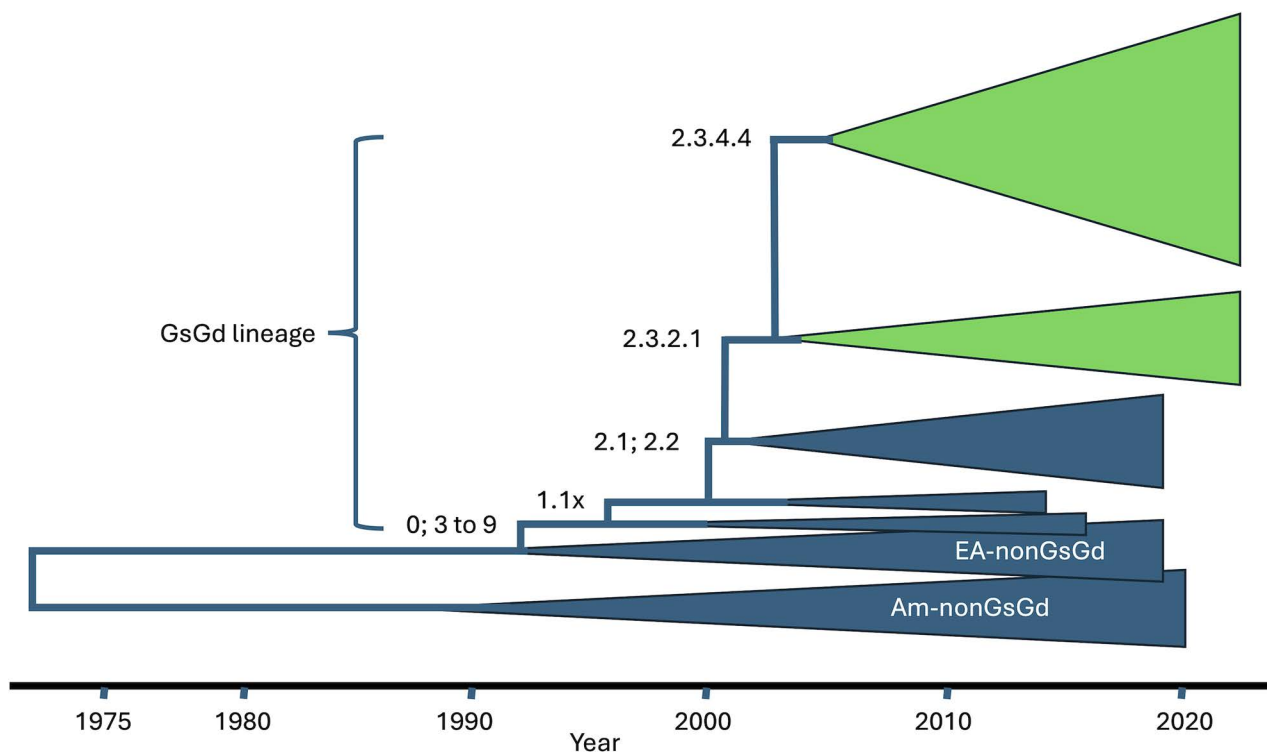


Figure 1—H5Nx lineage. The phylogeny is a simplified depiction of the most recent Nextstrain build for H5Nx by Louise Moncla (<https://nextstrain.org/flu/avian/h5nx/ha?d=tree&p=full>). Major clades are indicated at the root of triangular tree tips. Current circulating clades of H5Nx highly pathogenic avian influenza are shown in green triangles.

migratory stopover locations. Viral prevalence would then be low during the spring and summer periods before local epidemics increase prevalence in naïve hatch year birds during late summer in the northern hemisphere.^{16,17} Similar patterns may hold in the southern hemisphere but be organized around elements of the host bird's annual cycle specific to regional geography, climate, and habitat.¹⁸⁻²⁰ Much of the work to understand LPAI has focused on defining patterns of reassortment and viral evolution over time, in particular hosts, and in particular geographic regions, such as the Midwest^{21,22} or the Pacific flyway^{16,23} in the Americas. However, this is a very complicated endeavor in disease ecology as these environments are far from closed systems, and one of the characteristics that distinguishes influenza is a fairly broad host tropism. Therefore, while some subtypes of virus might be found predominantly in *Anatidae* species, and even more predominantly in specific species such as Mallard ducks, those hosts are subject to infection by new strains that enter the ecosystem. Moreover, the high mutation rate of influenza viral RNA genomes²⁴ and the ability and likelihood of viral reassortment between circulating strains mean that the system is already an extremely complex dynamic. Add the introduction of new viral strains and that complexity is increased. As H5 HPAI has spread throughout Eurasia and both into northern Europe and now into the Americas over the years, it has followed a different dynamic that is not well understood. In part, this is because it has been very difficult to identify and track the introduction of new strains on a local, let alone global scale. As such, good models for defining the epidemiology and ecology of novel strains such as H5 HPAI do not exist that are specific to influenza. What we do have is pieces of surveillance captured by a few different networks of scientists, governments, and agencies that are tracking the evolution and transmission of HPAI in avian and nonavian hosts where it cocirculates and frequently reassorts with another influenza A virus.^{1,25-27}

Mammalian Adaptation

The level of concern remains very high for transmission of HPAIv into agricultural poultry operations and much debate continues about the merits and pitfalls of vaccination and management strategies to prevent this.⁸ However, from the veterinary and human medicine standpoint, one of the most critical aspects being tracked is the spillover of current HPAI circulation into mammalian hosts and adaptation that may be associated with that spillover and onward transmission. Since January of 2023, several characteristics of this spillover have heightened concerns but require more research to understand. The first is the sheer number of animals and species in which spillover infection has been documented.^{25,28} The 2.3.4.4b viral lineage appears to infect multiple bird species more commonly than it has previously. Among these are a variety of raptors whose illness has proved highly visible due to their charismatic

nature and the efforts of multiple groups that respond to rescue and release injured and ill raptors back to the natural environment. However, in addition to raptors, many mammalian hosts have had infections with severe clinical signs, primarily neurologic, and death as a result.^{25,29}

Intermediate Host versus Direct Transmission

For a long time, the influenza research community has understood the role of intermediate hosts, such as swine, in introducing epidemic strains to human populations.^{30,31} Host tropism is governed in part by the ability of the HA molecule to bind sialic acid residues on host cell glycoproteins decorating the exterior surface of epidermal cells that become infected in the respiratory and gastrointestinal tracts. However, each host produces glycoproteins that are expressed on the exterior of the host cells in slightly different ways in different tissues. As a result, many LPAI circulating in the wild only bind well to cells of their primary host, for instance, avian cells, and not those of other animal hosts. Pigs, however, seem adept at hosting both some avian influenza strains and some human strains of influenza, which allows these hosts to serve as potential "intermediates" where mixing and reassortment of viral segments can result in a new virus better adapted to human infection but of a novel antigenicity more prone to epidemic spread and not covered by current vaccination from seasonal strains (**Figure 2**). In an alternative scenario for an intermediate host, another mammal's ability to host avian viruses can also provide time for additional mammalian-specific mutations or reassortment to be selected that predisposes the virus to further spillover and host expansion. It is believed this may explain some canine infections with influenza, as an example.³²

Over 2 years after its introduction to North America, it is unclear how transmission of the 2.3.4.4 viral lineage is occurring in most cases because it is difficult to document, and it is unclear how important any intermediate hosts may be to the probability that this lineage will become endemic in new hosts, particularly mammalian hosts. In much of the mammalian spillover that has occurred, a predator/prey relationship is assumed to be the route to infection, and little, if any, onward transmission in documented hosts has been observed. Although the implicated infected prey is rarely documented in a conclusive way, it is a logical conclusion for many situations where concomitant die-off is occurring in potential prey species. Such was the case for initial infections described in seal populations in the northeast US, where active documented die-offs of both ducks and gulls and related species were occurring in large numbers coincident with infection documented in a broad geographic area of seals.³³ However, it is equally unclear in the current pandemic circulation of HPAIv what role the contamination and persistence of the virus in the environment might take. Susceptible hosts, even in the absence of

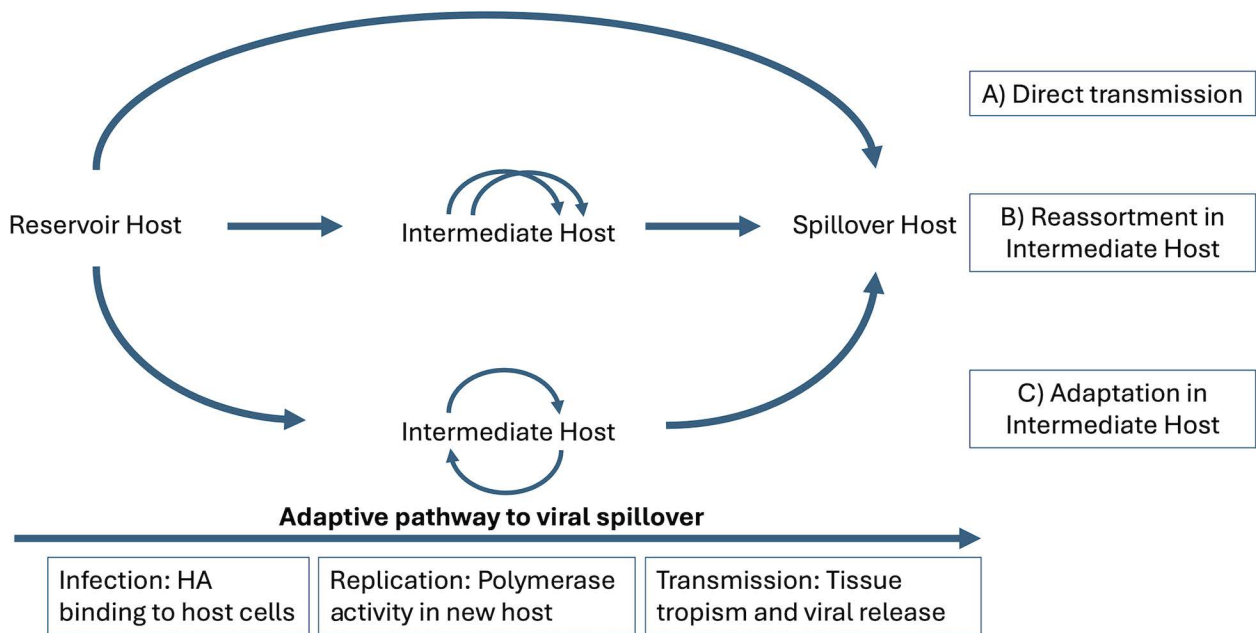


Figure 2—Possible routes of viral spillover from reservoir hosts. Three potential scenarios are depicted, including direct transmission (A), reassortment of multiple viruses in an intermediate host (B), and the adaptation of virus in an intermediate host prior to or consequent to spillover (C). Depicted at the bottom, regardless of route, spillover to novel animal hosts likely requires mutational selection impacting the ability of virus to (1) infect, (2) replicate, and (3) transmit to new hosts.

a predator/prey relationship, might become infected by contamination of drinking water or by grooming contaminated fur. The importance of environmental persistence has not been well addressed as a cause or consequence of the expansion of H5 HPAI circulation. Both laboratory and observational studies in the natural environment have established impacts of pH, temperature, and humidity on the persistence of viruses in both liquid and aerosol forms outside the host.³⁴ Viral and host factors, such as the lipid content of the viral envelope and the genetic characteristics of incorporated surface proteins as well as the agglutinating characteristics of the virus,^{35–38} may contribute to prolonged infectivity in the environment and to models of transmission and geographic expansion in epidemiological models.

Viral Phenotyping and Risk Assessment

The core of understanding the threat of circulating influenza virus, whether classified as LPAI or HPAI, is understanding how viral sequence or genotype translates to phenotype. For HPAIv, since its emergence in poultry populations, and particularly with its establishment in wild birds throughout Asia, major interest has been focused on the infectivity and pathogenicity of the circulating strains in model organisms in addition to their continuing evolution. In short, the circulating virus has caused human infection and death and continuing animal outbreaks. However, with the passage of time, knowledge, and public safety as well as the continued

coevolution of this lineage with its natural hosts, fewer spillover infections have occurred in humans and the virus appears to impact many of its natural avian hosts less severely. Nonetheless, a continued focus remains on determining what mutations are occurring or may occur that could be expected to change the infectivity, pathogenicity, and transmission of 2.3.4.4 or 2.3.3.1 strains in particular. A decade ago, studies in the ferret, a preferred model of human infection, demonstrated, with much controversy, that few changes were required to naturally isolated strains to allow it to transmit via aerosol or droplet transmission in cohoused animals.^{39–41} A small number of critical mutations have since been examined in specific strain backgrounds that allow significant changes in viral phenotype thought to be prerequisites to a naturally transmissible strain in mammalian hosts.

However informative these analyses have been in understanding the small distances potentially needed to be crossed in mutation space to drastically alter the possibility for emergence, they are limited by 2 confounding factors: (1) the continued evolution of the natural lineage, and, in a related way, (2) the changing background that specific genotypes are occurring in, either altered by mutation and drift over time and by the continual reassortment of genome segments. As such, the strains that have expanded globally now contain changes and present a different context that needs to be evaluated both with regard to mutations in past strains that are shown to have a significant impact and for novel mutations in the viral genome that are yet unexplored but that may ultimately lead to the correct viral genome context

to take advantage of fortuitous disease ecology and create the emergence of a viral epidemic in animal or human hosts.

New Approaches to Pandemic Preparedness Are Needed, Requiring New Research

As the H5 HPAI lineages continue to expand globally, even into the poles, the urgency to understand and be able to prepare for HPAI spillover and epidemic in a mammalian host is increasing. There is still the problem of the incredible diversity of potential pandemic viruses circulating and the ability to translate viral genotypes into understandable or predictable viral phenotypes. This approach will require the cooperative expertise of numerous researchers working together to narrow an unmanageable threat to an understood analysis of risk (**Figure 3**). This pipeline starts with expanded efforts in surveillance that are needed to characterize and have knowledge of the extent and intensity of viral circulation to know the diversity that exists. Because we have a substantial amount of research and knowledge about prior strains and circulating viruses, a second step entails the phylogenetic and other modeling approaches to define the relationship of viral isolates. These first 2 steps are a matter of mostly implementation; the

third step requires the combination of a suite of phenotyping assays that could be done in high throughput and rapidly enough to take a still large list of potential and real viral isolates down to a manageable scale that can be understood as phenotypes in animal models. Using the known history of viral spillover as well as new approaches such as artificial intelligence, new protocols can help us build models that will help to understand the risk of viral spillover and the potential early signs to watch for with continued circulation of the virus.

Animal Models and Comparative Pathology

The other major challenge concerns the last step of the approach outlined above, animal models of disease. The problem is a simple one in that it is very easy to draw analogies between disease states in different hosts, but it is very hard to translate, with confidence, the impacts of novel disease pathology between one host and another. However, there is currently no alternative approach. As such, understanding the comparative pathology more completely, to an extent that allows confident modeling between model animals and humans or other hosts, is critical. Much more research in both infection as well as immune response and pathology is critically needed.

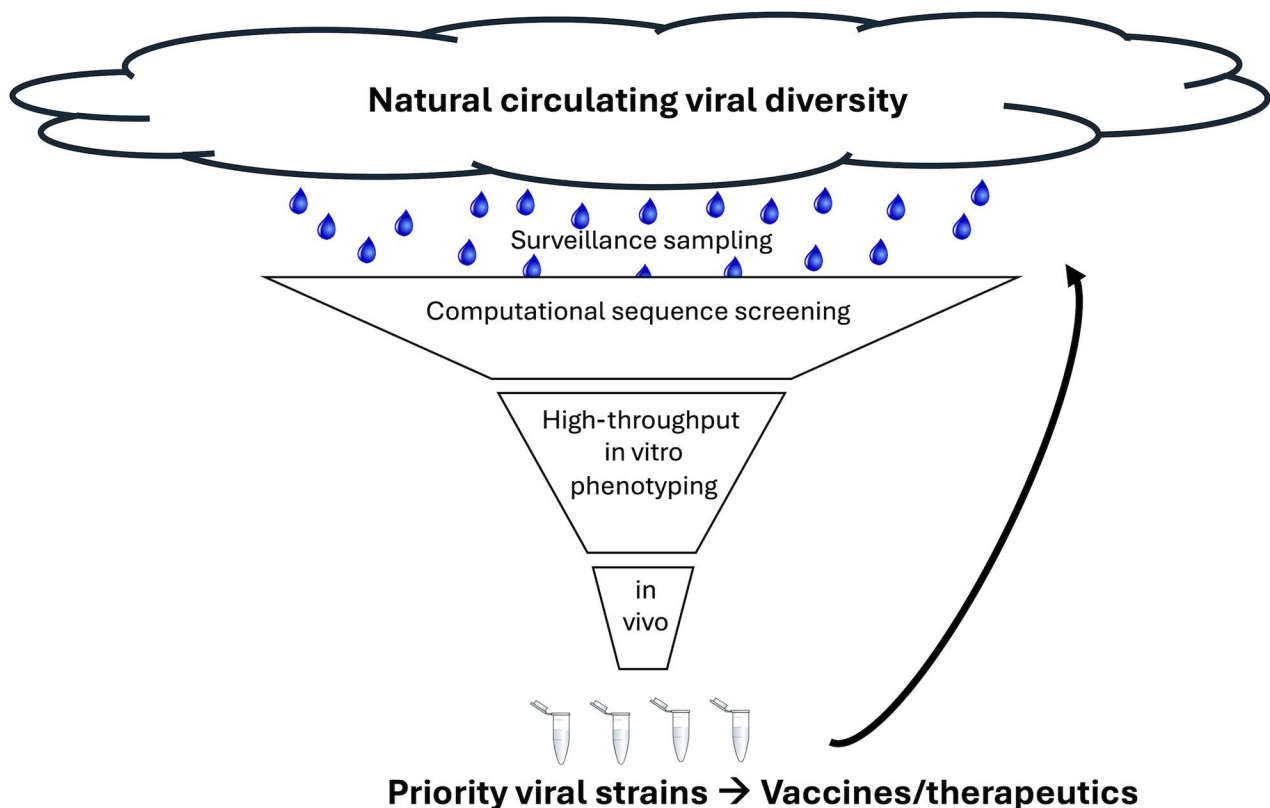


Figure 3—Optimizing the pipeline for characterizing the natural diversity of highly pathogenic avian influenza in wild reservoirs. Pipeline includes the recurring sequential use of surveillance and computational methods to select representative viruses, high-throughput in vitro methods to phenotype viral strains, and in vivo experiments to phenotype candidate strains.

Conclusions

The current circulation of evolving lineages of HPAIv is challenging our understanding and assessment of the threat of this virus in a One Health context. Novel neurogenic phenotypes in novel hosts, increased frequency of spillover into mammalian hosts, and an ever-expanding diversity of H5Nx viral genotypes circulating in animal reservoirs in new ecosystems have demonstrated the limitations of current understanding. Research is focused on understanding the potential of viruses to spill over through (1) better understanding of virus ecology in diverse hosts, and (2) characterizing the relationship between viral genotype and viral phenotype in a diverse background. Breakthroughs that will help predict and limit the impact of HPAI on animals, humans, and the environment will depend on the implementation of new approaches to characterize global diversity through analysis pipelines that use computational models and machine learning, high-throughput in vitro phenotyping, and limited in vivo experiments to assess HPAI.

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Disclosures

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