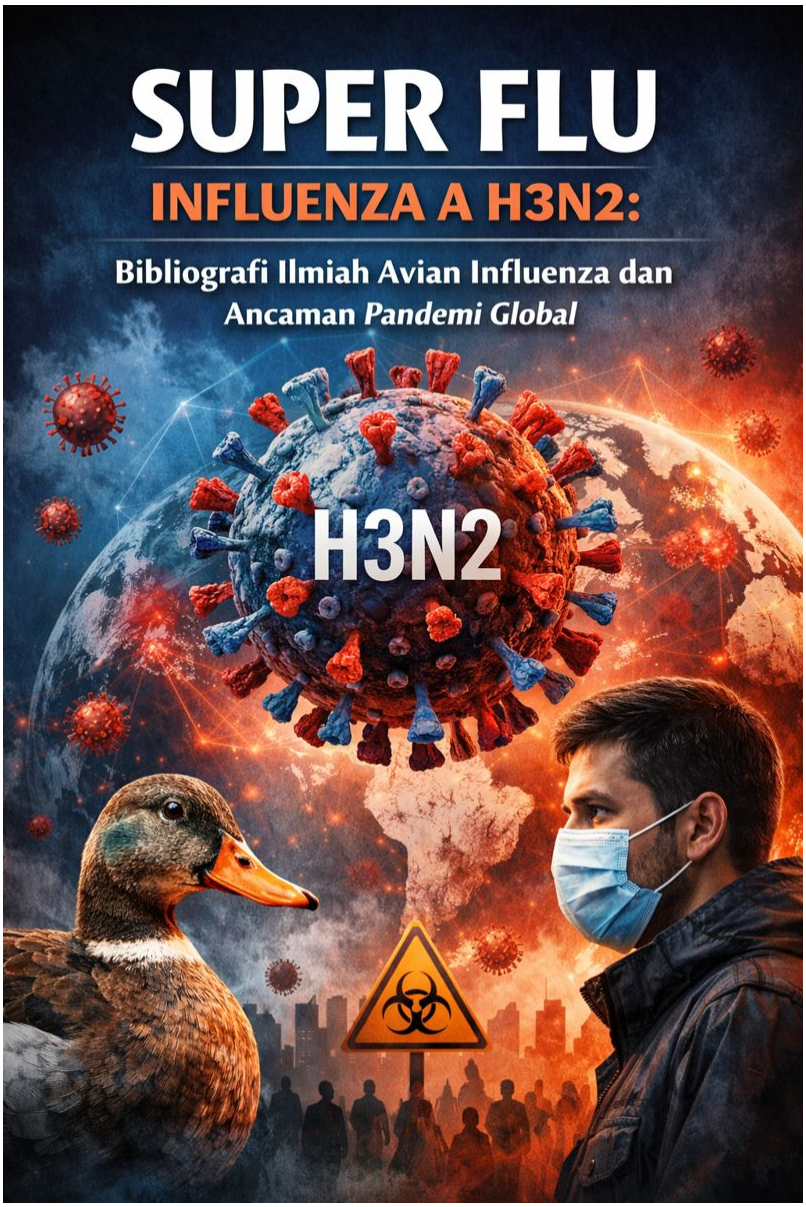
SUPER FLU INFLUENZA A H3N2: BIBLIOGRAFI ILMIAH AVIAN INFLUENZA DAN ANCAMAN PANDEMI GLOBAL



PERPUSTAKAAN BALAI BESAR PERAKITAN DAN MODERNISASI VETERINER

BADAN PERAKITAN DAN MODERNISASI PERTANIAN

KEMENTERIAN PERTANIAN

2026

KATA PENGANTAR

Puji syukur ke hadirat Tuhan Yang Maha Esa atas rahmat dan karunia-Nya sehingga penyusunan bibliografi berjudul **"Super Flu Influenza A H3N2: Bibliografi Ilmiah Avian Influenza dan Ancaman Pandemi Global"** dapat diselesaikan dengan baik. Bibliografi ini disusun sebagai upaya sistematis untuk menghimpun dan mendokumentasikan berbagai sumber ilmiah yang membahas virus influenza A, khususnya subtipe **H3N2**, serta subtipe avian influenza lainnya yang memiliki peran penting dalam dinamika evolusi virus dan potensi terjadinya pandemi global.

Istilah *super flu* dalam konteks bibliografi ini digunakan untuk menggambarkan virus influenza dengan karakteristik adaptasi tinggi, kemampuan reassortment genetik, transmisi lintas spesies, serta potensi peningkatan patogenisitas dan penyebaran luas pada populasi manusia maupun hewan. Oleh karena itu, literatur yang dikompilasi tidak hanya mencakup H3N2, tetapi juga berbagai subtipe influenza avian lainnya yang relevan dalam aspek virologi, epidemiologi, imunologi, surveilans, deteksi diagnostik, pengembangan vaksin, dan terapi antivirus.

Daftar pustaka dalam bibliografi ini berasal dari berbagai penelitian eksperimental, studi lapangan, analisis genomik, serta kajian risiko kesehatan masyarakat yang menyoroti interaksi kompleks antara virus influenza, inang hewan, dan manusia. Kompilasi ini diharapkan dapat memberikan gambaran komprehensif mengenai perkembangan ilmiah terkini sekaligus meningkatkan kewaspadaan terhadap ancaman influenza yang berpotensi berkembang menjadi pandemi.

Penyusunan bibliografi ini diharapkan dapat menjadi sumber rujukan yang bermanfaat bagi mahasiswa, peneliti, akademisi, tenaga kesehatan, serta pemangku kebijakan dalam memahami fenomena *super flu* dan tantangan global yang ditimbulkannya. Selain itu, bibliografi ini juga diharapkan dapat mendukung penelitian lanjutan serta pengambilan keputusan berbasis bukti ilmiah dalam upaya pencegahan dan pengendalian influenza.

Penulis menyadari bahwa bibliografi ini masih memiliki keterbatasan. Oleh karena itu, kritik dan saran yang bersifat membangun sangat diharapkan guna penyempurnaan di masa mendatang. Semoga karya ini dapat memberikan kontribusi positif bagi pengembangan ilmu pengetahuan dan upaya perlindungan kesehatan global.

DAFTAR ISI

1. Lei Shi, Yuekun Lang, Sawrab Roy, Zhenyu Shen, Dipali Gupta, Chao Dai, Muhammad Afnan Khalid, William J. Mitchell, Shuping Zhang, Richard Webby, Juergen Richt, Wenjun Ma,**Outcomes of experimental infection of calves with swine influenza H3N2 virus**,mBio,Volume 16, Issue 7, 2025,https://doi.org/10.1128/mbio.03957-24.

ABSTRACT

Unprecedented outbreaks caused by the H5N1 highly pathogenic avian influenza virus (HPAIV) among dairy cows in the United States have raised significant concerns. Whether other subtypes of influenza A viruses (IAVs) can infect and transmit in cattle remains largely unknown. Herein, we infected cattle respiratory and mammary gland cells with different IAVs and two groups of Holstein calves intranasally or orally with a swine H3N2 virus to determine their susceptibility. Naive calves were co-housed with infected animals to investigate virus transmission. Results showed that tested swine and avian IAVs could infect cattle primary nasal turbinate and tracheal epithelial cells, as well as immortalized mammary gland epithelial cells and fibroblasts. No obvious clinical signs, including fever, were observed in infected and contact calves, but macroscopic lung lesions were found in necropsied animals in both groups on day 5 post-infection. Viral shedding was detected in three out of four nasally infected calves but not in orally infected or the two groups of contact animals. Interestingly, viral RNA and antigen could not be detected in all tissues from individual necropsied animals from either infection group, but viral RNA and sequences were detected in serum samples of two nasally infected calves on day 7 post-infection, not on other days and in other animals. Additionally, only the nasally infected animals seroconverted. Our results indicate that in addition to H5N1 HPAIV, swine H3N2 virus can infect cattle but does not transmit efficiently among them, suggesting that other subtypes of IAVs could infect and replicate in cattle. IMPORTANCE Highly pathogenic avian influenza H5N1 virus outbreaks in U.S. dairy herds have raised questions about whether other subtypes of influenza A viruses (IAVs) can infect and transmit in cattle. In this study, we investigated the susceptibility and infection of different IAVs in bovine primary and immortalized cells and Holstein calves. Results showed that avian H5N1 and H9N2, and swine H3N2 IAVs could infect beef cattle primary nasal turbinate and tracheal epithelial cells, as well as immortalized mammary gland epithelial cells and fibroblasts. Moreover, the swine H3N2 could infect the calves through intranasal infection, but not through oral infection, despite no obvious clinical signs and efficient transmission being observed. Our results demonstrate that other subtypes of IAVs can infect cattle and might pose threats to public and animal health.

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observed. Our results demonstrate that other subtypes of IAVs can infect cattle and might pose threats to public and animal health.

Keywords: cattle respiratory and mammary gland cells; calves; swine and avian influenza viruses; infection; transmission

2. Fei-fei Ge, Li-ping Shen, De-quan Yang, Hai-xiao Shen, Xin Li, Jian Liu, Jian Wang, Hongjin Zhao**, Robust and sustained immunity in beagles following one single nasal administration of H3N2 canine influenza virus-like particle**, Vaccine: X, Volume 27, 2025, https://doi.org/10.1016/j.jvacx.2025.100739.

ABSTRACT

In this study, we used baculovirus to express hemagglutinin (HA) and neuraminidase (NA) to prepare a novel genotype of H3N2 canine influenza virus particles (VLPs). The effectiveness of the H3N2 VLP vaccine was evaluated by detecting HI antibodies, the antiviral protection rate, antibody persistence and anatomical examination of the lungs.A challenge model has been established in a previous study for the study of canine influenza virus-like particle vaccines. A/Canine/Shanghai/0103/2019, with a challenge dose of 106 EID50, infects 10-week-old healthy beagle dogs through nasal instillation and can cause severe clinical symptoms. Using a single dose of VLP vaccine for beagle dogs, the vaccine was tested at titers of 26 intranasally and 26 intramuscularly. One week after a single immunization, the HI titer promptly reached 28 among the immunized groups. The duration of antibody can persist for four months. We differentiated between CD4+ and CD8+ T cells in the peripheral blood. Four weeks after the single immunization, all beagles except those in the noninfected and nonimmunized groups were intranasally challenged with live H3N2 virus (1 × 106 EID50). All immunized beagles shed no virus at d 1–4 post-challenge. After the challenge, the placebo control beagles shed the virus on d 1 post-challenge (105.85±0.071 EID50). An anatomical examination of the lungs revealed that visible lesions were rarely detected in the lungs of the nasal immunization group, and the lungs were as healthy as those of the noninfected and nonimmunized groups were. The lung surfaces presented visible bleeding spots in the intramuscular immunization group and placebo-control group. Their effectiveness will provide a scientific basis for the promotion and use of these products.

Keywords: Novel genotype; H3N2 canine influenza virus particles; Intranasally; Duration of antibody; CD4+ and CD8+ T cells; Effectiveness

3. Mingkun Wu, Liru Guo, Mei Kong, Ming Zou, Xiaochang Liu, Xiaoyan Li, **Epidemiological and genomic surveillance of influenza A virus** (pdm09 H1N1 and H3N2) strains from 2017 to 2025 in Tianjin, China, Virologica Sinica, Volume 40, Issue 4, 2025, https://doi.org/10.1016/j.virs.2025.07.011.

ABSTRACT

Influenza A virus (IAV) remains a global public health concern, causing influenza-like illness and severe respiratory tract infections. Two major subtypes, A/pdm09 H1N1 and A/H3N2, circulate globally, and their epidemics are influenced by multiple factors, especially during the COVID-19 pandemic. Based on data from the National Influenza Surveillance Program in China, we analyzed the epidemiological and genomic data in Tianjin collected from 2017 to 2025. A total of 77,473 throat swabs were collected, of which 9144 were IAV-positive. The A/pdm09 H1N1 and A/H3N2 lineages exhibited distinct epidemics across different influenza seasons, with a decline in cases observed during the COVID-19 pandemic. We sequenced the genomes of 128 A/pdm09 H1N1 and 113 A/H3N2 clinical isolates and characterized their temporal evolution and genetic diversity using time-scaled phylogenetic analysis. Additionally, we conducted a genetic risk evaluation of the hemagglutinin and neuraminidase segments, identifying key amino acid residues associated with viral adaptation, transmissibility, virulence, and drug resistance. Moreover, no antigenic variants were found in clinical isolates during the recent influenza seasons, though reduced sensitivity to oseltamivir and zanamivir was observed in individual strains. Our surveillance highlights the epidemiology and evolution of IAV before and after the COVID-19 pandemic in Tianjin.

Keywords: Epidemiology; Influenza A virus (IAV); Next-generation sequencing; Antigenicity; Drug resistance

4. Anne P. Werner, Cosette G. Schneider, Elgin H. Akin, Juliahna Hayes, Katherine Z. J. Fenstermacher, Richard E. Rothman, Lynda Coughlan, Andrew Pekosz**, Low levels of influenza H5N1 HA and NA antibodies in the human population are boosted** by seasonal H1N1 infection but not by H3N2 infection or influenza vaccination, mBio, Volume 16, Issue 12, 2025, https://doi.org/10.1128/mbio.02145-25.

ABSTRACT

An increase in the number of human cases of influenza A/H5N1 infection in the USA has raised concerns about the pandemic potential of the virus. Pre-existing population immunity is a key determinant for risk assessment and pandemic potential for any virus. Antibody responses against the bovine A/H5N1 hemagglutinin (HA) and neuraminidase (NA) proteins were measured among a population of influenza-vaccinated or influenza-infected individuals. Modest titers of bovine A/H5N1 HA-binding antibodies and low to undetectable neutralizing antibody titers were detected in a cohort of 73 individuals. Conversely, bovine A/H5N1 NA-binding and neuraminidase-inhibiting antibody titers were comparable to those against a human A/H1N1 NA at baseline. Seasonal influenza vaccination failed to significantly increase antibody titers against both HA and NA glycoproteins of bovine A/H5N1. Recent infection with

human A/H1N1 but not A/H3N2 viruses induced significant increases in bovine A/H5N1-neutralizing antibody, as well as increases in NA-binding and NA-inhibiting antibodies to bovine A/H5N1 NA. While the degree of protection afforded by these A/H5N1 cross-reactive antibodies is not known, incorporating NA or enhancing current seasonal vaccine formulations to increase NA-specific antibody titers may increase antibody breadth and protection against both seasonal and pandemic influenza viruses. IMPORTANCE A/H5N1 influenza A viruses continue to pose a pandemic threat to humans. Recent infection of dairy cattle and poultry with A/H5N1 in the USA has magnified that concern. We determined the level of antibodies that recognize A/H5N1 hemagglutinin (HA) and neuraminidase (NA) proteins in a population in Baltimore, MD. We show that while low levels of H5 HA-binding and A/H5N1-neutralizing antibodies are present, there is a significantly stronger recognition of bovine N1 NA. Vaccines that target the N1 NA protein may induce protective antibody responses in humans due to the presence of cross-reactive human N1 NA antibodies.

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Keywords: H5N1; neuraminidase; hemagglutinin; population immunity; neutralizing antibody; neuraminidase inhibiting antibody

5. Pengfei Wang, Linlin Yan, Jing Wang, Shoukui Hu, Fan Zhao**, Utilizing multiplex reverse transcription-multiple cross displacement amplification-lateral flow biosensor technology for detecting H1N1, H3N2 and H7N9 influenza A virus subtypes**, Journal of Virological Methods, Volume 338, 2025, https://doi.org/10.1016/j.jviromet.2025.115235.

ABSTRACT

Respiratory infections caused by influenza A viruses (IAVs) pose a global threat annually, leading to significant mortality in severe cases. IAVs are classified into various subtypes, and a combination of multiplex reverse transcription-multiple cross displacement amplification (mRT-MCDA) and lateral flow biosensor (LFB) has been developed to detect three subtypes (H1N1, H3N2, and H7N9) that have been frequently observed in recent years. This technology simultaneously reverse transcribes and amplifies two target genes, hemagglutinin (HA) and neuraminidase (NA), at a constant temperature of 63 °C in just 40 min. The final results can be read using a dual-channel LFB. In practice, the entire process—from sample collection, RNA extraction, and mRT-MCDA amplification to result interpretation—can be completed in less than 1 h. This method is named "multiplex reverse transcription-multiple cross displacement amplification-lateral flow biosensor" (IAVs-mRT-MCDA-LFB) technology. The

IAVs-mRT-MCDA-LFB technology demonstrated higher sensitivity compared to conventional reverse transcription-polymerase chain reaction (RT-PCR) and showed no cross-reactivity with other genes present in the samples. Therefore, the IAVs-mRT-MCDA-LFB technique developed in this study is a highly valuable molecular diagnostic tool for detecting IAV-subtypes due to its simplicity, time efficiency, cost-effectiveness, and high specificity and sensitivity.

Keywords: Influenza A viruses; Multiplex; Reverse transcription; Multiple cross displacement amplification; Lateral flow biosensor

6. Jing Yang, Shufa Zheng, Ju Sun, Haibo Wu, Dan Zhang, Yanjun Wang, Tian Tian, Linwei Zhu, Zhigang Wu, Lanjuan Li, George F. Gao, Yuhai Bi, Hangping Yao, **A human-infecting H10N5 avian influenza virus: Clinical features, virus reassortment, receptor-binding affinity, and possible transmission routes**, Journal of Infection, Volume 90, Issue 4, 2025, https://doi.org/10.1016/j.jinf.2025.106456.

ABSTRACT

Background

In late 2023, the first human case caused by an H10N5 avian influenza virus (AIV) was diagnosed in China. H10Ny AIVs have been identified in various poultry and wild birds in Eurasia, the Americas, and Oceania.

Methods

We analyzed the clinical data of the H10N5 AIV-infected patient, isolated the virus, and evaluated the virus receptor-binding properties together with the H10N8 and H10N3 AIVs identified in humans and poultry. The genomic data of the human-infecting H10N5 strain and avian H10Ny AIVs (n = 48, including 16 strains of H10N3 and 2 strains of H10N8) from live poultry markets in China, during 2019–2021, were sequenced. We inferred the genetic origin and spread pattern of the H10N5 AIV using the phylodynamic methods. In addition, given all available nucleotide sequences, the spatial-temporal dynamics, host distribution, and the maximum-likelihood phylogenies of global H10 AIVs were reconstructed.

Findings

The first H10N5 AIV-infected human case co-infected with seasonal influenza H3N2 virus was identified. Unfortunately, the patient died after systematic treatments. The H10N5 virus predominantly bound avian-type receptor, without any known mammalian-adapted mutations. Phylodynamic inference indicated that the H10N5 AIV was generated by multiple reassortments among viruses from Korea and Japan, central Asia, and China in late 2022, acquiring the seven gene segments from H10N7 or other low pathogenic AIVs in wild Anseriformes, except for the PA gene from H5N2 AIVs in Domestic Anseriformes. The HA gene of the H10N5 virus belongs to the North American lineage, which was probably introduced into Asia by migratory birds, subsequently forming local circulation.

Interpretation

Unlike the human-infecting H10N3 and H10N8 AIVs acquiring six internal protein-coding genes from H9N2 AIVs in domestic poultry, the human-infecting H10N5 AIV was generated through multiple reassortments among viruses mainly carried by wild Anseriformes. Furthermore, worldwide distribution, inter-continental transmission, and genetic exchanges between Eurasian and North American lineages call for more concerns about influenza surveillance on H10Ny AIVs, especially in the flyway overlapping areas.

Keywords: H10N5 avian influenza virus; Clinical features; Zoonosis; Genetic tracing; Phylodynamics; Novel reassortant

7. Han Li, Qi Tong, Mengyan Tao, Jixiang Li, Haili Yu, Qiqi Han, Jiancheng Wu, Riguo Lan, Jingjing Han, Haoyu Chang, Yan Li, Juan Pu, Yipeng Sun, Litao Liu, Yajin Qu, Quanlin Li, Lu Lu, Jinhua Liu, Honglei Sun, **Assessment of the public health risk of novel reassortant H3N3 avian influenza viruses that emerged in chickens**, mBio, Volume 16, Issue 7, 2025, https://doi.org/10.1128/mbio.00677-25.

ABSTRACT

Influenza A (H3N2) viruses are historically responsible for the 1968 Hong Kong flu pandemic. Since then, H3N2 has continued to circulate as a seasonal influenza virus in humans. Public health concerns were raised in 2022 when human infections with novel reassortant H3N8 influenza viruses originating from chickens were first reported in China. Here, we conducted a systematic surveillance of H3 avian influenza viruses (AIVs) circulating in poultry and assessed the public health risk of emergent H3 reassortants. We found that H3 AIVs were prevalent in both ducks and chickens. Notably, in December 2022, a novel chicken-derived H3N3 subtype virus was identified, which gradually replaced the previously predominant H3N8 virus and became prevalent in chickens. Genetic analysis demonstrated that the novel H3N3 virus is a triple-reassortment strain with the H3 gene segment from chicken H3N8 virus, the N3 gene segment from the H10N3 virus, and internal gene segments derived from H9N2 viruses. Compared with chicken H3N8 and duck H3N3 viruses, the novel chicken H3N3 viruses produced higher yields and induced greater pathogenicity in human respiratory epithelial cells and mammalian models (mouse and ferret). Importantly, the chicken H3N3 viruses could be transmitted efficiently between ferrets through direct contact. The polymerase activity of the chicken H3N3 viruses in mammalian cells was markedly increased by the PA gene originating from the H9N2 virus. Our findings indicate that the circulation of novel chicken H3N3 viruses poses a threat to both the poultry industry and human public health. IMPORTANCE The H3Ny subtype influenza A virus can infect a wide range of hosts. In addition to circulating among wild birds and poultry, the virus can also infect humans and a variety of mammals. Here, we found that H3Ny subtype AIVs were widely prevalent in domestic chickens and ducks. Novel H3N3 reassortant viruses emerged as a result of the genetic reassortment of the chicken-derived H3N8 AIVs with H10N3 and H9N2 AIVs. The novel H3N3 subtype AIVs are gradually displacing H3N8 AIVs and becoming prevalent in chickens.

Furthermore, these novel H3N3 AIVs exhibited enhanced infection ability and efficient transmissibility in mammalian models, indicating a growing potential public health risk.

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Keywords: H3N3; avian influenza viruses; genetic reassortment; public health

8. Ping Wang, Han Wu, Jiamin Fu, Jun Zhang, Linfang Cheng, Fumin Liu, Hangping Yao, Nanping Wu, Haibo Wu**, Rapid and sensitive time-resolved fluorescence immunochromatographic strip for detecting H10 subtype avian influenza virus**, Poultry Science, Volume 105, Issue 1, 2026, https://doi.org/10.1016/j.psj.2025.106123.

ABSTRACT

Human infections with the H10 subtype avian influenza virus (AIV) have been reported in recent years, raising concern about potential human-to-human transmission. Effective field detection methods are essential for monitoring and controlling the spread of this virus. The objective of this study was to develop a rapid, highly sensitive, and specific detection system for the H10 subtype AIV. Two monoclonal antibodies targeting the hemagglutinin (HA) protein of H10 AIV were generated and characterized. One antibody was conjugated to europium-chelate fluorescent nanospheres and used as a tracer, while the other was immobilized on the test line to capture antigen. A time-resolved fluorescence immunochromatographic strip was then constructed and evaluated for analytical performance. The assay achieved a detection limit of 0.015 hemagglutination units for virus-containing allantoic fluid and 1.1 ng/mL for purified HA protein, which represents a substantial improvement in sensitivity compared with conventional immunochromatographic assays. The test correctly identified all H10 subtype strains examined, including H10N2, H10N3, H10N5, H10N7, and H10N8, and showed no cross-reactivity with nine other subtype AIVs or seven non-influenza avian pathogens. Reproducibility was high, with relative standard deviations below 10% across repeated assays. Receiver operating characteristic analysis yielded areas under the curve of 0.9896 and 0.9722 for virus- and protein-based evaluations, respectively, confirming excellent diagnostic accuracy. Field evaluation with 174 avian samples, including cloacal swabs, throat swabs, and fecal samples, demonstrated 100% concordance with real-time polymerase chain reaction. The entire test procedure was completed within 15 minutes, requiring minimal equipment and technical expertise. In conclusion, the developed time-resolved fluorescence immunochromatographic strip provides a rapid, sensitive, and highly specific tool for detecting the H10 subtype AIV. Its superior performance and ease of use make it well suited

for routine surveillance and early warning in poultry farms and live bird markets, thereby supporting disease control and protecting public health.

Keywords: H10 avian influenza virus (AIV); Monoclonal antibody; Time-resolved fluorescence; Immunochromatographic strip; Rapid detection

9. Chaeeun Kim, Thi Hoai Phan, Anh Duc Truong, Yeong Ho Hong, **Exosomes derived from avian influenza virus-infected chickens modulate host immune responses**, Poultry Science, 2026, https://doi.org/10.1016/j.psj.2026.106386.

ABSTRACT

Exosomes are emerging as key mediators of host–pathogen interactions, particularly as carriers of viral components during infection. This study aims to examine the immunomodulatory effects of serum-derived exosomes from Brown Leghorn chickens infected with low pathogenic avian influenza virus (LPAIV) or highly pathogenic avian influenza virus (HPAIV). These exosomes (CTRL-EXO [noninfected], LPAIV-EXO, and HPAIV-EXO) were intramuscularly injected into naïve chickens, after which tissues and serum were collected. Cytokine gene expression in immune-related tissues (lung, spleen, and trachea) was quantified through reverse transcription-quantitative polymerase chain reaction to evaluate the immune response. Unlike the lung and trachea, the spleen showed the strongest immune response following exosome injection, associated with elevated antiviral cytokines and interferons in the AIV-exosome group. In parallel, these exosomes were applied to chicken macrophage HD11 cells to determine cellular uptake and cytokine expression using reverse transcription-quantitative polymerase chain reaction. Furthermore, immunocytochemistry was performed to detect exosome-delivered viral nucleoprotein and nonstructural protein 1 proteins in HD11 cells. LPAIV-EXO induced the strongest immune activation, evidenced by increased cytokine expression and immunochemical detection of viral proteins. Collectively, these findings indicate that AIV-derived exosomes modulate host immune responses in vivo and in vitro, underscoring their potential in immune regulation and vaccine development.

Keywords: Exosome; avian influenza virus; cytokine; viral component; chicken

10. Janak Dhakal, Sushant Bhat, Joe James, Richard Y. Otwey, Sandesh Chapagain, Parminder Singh, **Highly Pathogenic Avian Influenza (HPAI) H5N1 in Raw Pet Foods and Milk: A Growing Threat to both Companion Animals and Human Health, and Potential Raw Pet Food Industry Liability**, Journal of Food Protection, Volume 88, Issue 11, 2025, https://doi.org/10.1016/j.jfp.2025.100628.

ABSTRACT

The increasing popularity of raw meat-based diets (RMBDs) and raw milk feeding in companion animals presents a growing concern for zoonotic disease transmission. Recent evidence has demonstrated that these products can serve as vehicles for highly pathogenic avian influenza (HPAI) H5N1, an emergent viral threat with a host range from birds, dairy

cattle, and pets to humans. Since the emergence of clade 2.3.4.4b in 2020, HPAI H5N1 has caused widespread outbreaks in poultry, wild birds, and mammals, including dairy cattle and cats. Transmission to pets has been linked to ingestion of contaminated raw pet food and unpasteurized milk. Notably, multiple outbreaks in cats across Europe, Asia, and North America have been associated with raw pet food products, while recent U.S. cases confirm direct viral transmission from infected pet food, raw milk, and colostrum. Experimental studies have also supported the plausibility of gastrointestinal and respiratory routes of infection in cats and dogs, with felines appearing particularly susceptible, often exhibiting severe clinical disease and high mortality. A number of documented recalls of H5N1-contaminated raw pet food and raw milk in the US underscore the persistence of infectious viruses in cold-stored food products and highlight the risks of feeding raw diets. Although pet-to-human transmission of the HPAI H5N1 virus has not been reported yet, cat-to-human transmission of the H7N2 influenza virus has been reported in the USA. This review presents current evidence on H5N1 in RMBDs and raw milk, its epidemiology in companion animals, outbreaks, and the health implications among pets and humans. By raising awareness among pet owners, industry stakeholders, and veterinarians, this paper highlights the immediate need for stringent surveillance and improved biosecurity in raw food supply chains to minimize viral transmission risks, thereby safeguarding pet health and curb the potential spillover to humans.

Keywords: Cats; Dogs; H5N1; High Pathogenicity Avian Influenza (HPAI); Highly Pathogenic Avian Influenza (HPAI); Raw milk; Raw pet food; Zoonotic transmission

11. Mirjam B.H.M. Duijvestijn, Nancy N.M.P. Schuurman, Johannes C.M. Vernooij, Marian J. Broekhuizen, Erwin de Bruin, Benine S. Carrière, Judith M.A. van den Brand, Jaap A. Wagenaar, Frank J.M. van Kuppeveld, Herman F. Egberink, Cornelis A.M. de Haan, Josanne H. Verhagen, **Hunting-training dogs and companion dogs in the Netherlands are frequently exposed to highly pathogenic avian influenza (HPAI H5) and human H1N1 virus**, 2021–2023, One Health, Volume 21, 2025, https://doi.org/10.1016/j.onehlt.2025.101134.

ABSTRACT

Dogs are susceptible to the currently circulating highly pathogenic avian influenza (HPAI) H5 and human H1N1pdm2009 (pandemic H1N1) viruses, yet little is known about the extent to which dogs are exposed to both these viruses. Here we investigated HPAI H5 and human H1N1pdm2009 virus exposure in domestic dogs–including dogs that participated in hunting-training–and investigated lifestyle factors associated with HPAI H5 virus exposure. We screened sera from 538 dogs, sampled between 2021 and 2023, for influenza A virus antibodies, using ELISA and hemagglutination inhibition assays (HAIs). We analyzed lung tissue and (naso)pharyngeal swabs for influenza A viruses using RT-qPCR. Seropositivity to HPAI H5 virus was more frequent (13.3 %) in hunting-training dogs than in companion dogs with unknown bird contact (3.7 %). In contrast, seropositivity to H1N1pdm2009 was more frequent in companion dogs (7.1 %) than in hunting-training dogs (0.7 %). Based on owner

questionnaires, seropositivity to HPAI H5 by ELISA in hunting-training dogs was significantly associated with recent bird contact in/near water (odds ratio 6.9). No influenza A viruses were detected in 207 necropsy dogs and 180 (hunting) dogs. Our findings suggest that dogs are frequently exposed to zoonotic influenza A viruses, and we recommend dog owners to avoid dog contact with sick/dead birds.

Keywords: Serosurveillance; Zoonosis; Pandemic preparedness; Avian influenza; Canine; Antibodies; Europe

12. Li-guo Liang, Ping Wang, Jiamin Fu, Linwei Zhu, Linfang Cheng, Fumin Liu, Nanping Wu, Lihua Xu, Hangping Yao, Haibo Wu,**Portable lab-on-a-chip platform enabling multiplex recombinant enzyme polymerase amplification detection of H5/H7/H10 avian influenza virus subtypes**, Poultry Science, Volume 104, Issue 9, 2025, https://doi.org/10.1016/j.psj.2025.105463.

ABSTRACT

The zoonotic nature of influenza pathogens creates substantial health security risks, jeopardizing the welfare of interconnected human and animal ecosystems. The H5/H7/H10 avian influenza virus (AIV) variants demonstrate persistent endemicity in poultry reservoirs and recurrent zoonotic jumps precipitating fatal human infections. Therefore, the innovation of multiplex diagnostic platforms integrating expedited processing, enhanced sensitivity, and subtype-specific discrimination has emerged as a pivotal strategy to curb epidemiological escalation. This research introduces a temperature-controlled nucleic acid detection platform utilizing microfluidic technology, enabling concurrent differentiation of H5, H7, and H10 AIV subtypes. Based on the conserved sequences of the hemagglutinin (HA) gene of H5, H7, and H10 AIVs, three sets of primers and probes specific to the subtypes were developed. These were then combined with microfluidic microarray technology and recombinant enzyme polymerase amplification. This combination aimed to create a method for the simultaneous detection of H5, H7, and H10 AIVs for differential diagnostic purposes. The method was distinguished by its specificity, sensitivity, accuracy, and its ability to detect these viruses in clinical samples. The specificity of the method showed that it could detect all strains of H5, H7 and H10 AIVs at the same time, with no cross-reactivity with other subtype influenza viruses or other avian pathogens. The sensitivity results showed that the assay could still detect the three AIV target genes simultaneously at a concentration of 2 copies per reaction. The results of this method for 100 clinical samples were consistent with those produced by quantitative PCR. This integrated detection system for H5/H7/H10 AIV differentiation exhibits exceptional specificity, enhanced sensitivity, rapid turnaround, and streamlined operational procedures, representing a viable solution for prompt pathogen identification during outbreaks.

Keywords: Avian influenza virus; Subtype; Detection; Microfluidic microarray; Recombinant enzyme polymerase amplification

13. Jiaying Yang, Xiaojing Chen, Xiyan Li, Ye Zhang, Jia Liu, Min Tan, Hong Bo, Wenfei Zhu, Lei Yang, Dayan Wang, Yuelong Shu, **Global spread of H3 subtype avian influenza viruses with an accelerated evolution after interspecies transmission**, Journal of Infection, Volume 91, Issue 2, 2025, https://doi.org/10.1016/j.jinf.2025.106542.

ABSTRACT

The H3 subtype avian influenza virus (AIV) has been widely spread in birds and is known as a natural source of mammalian influenza viruses. Based on data from public databases and our surveillance data, we analyzed the ecology, evolution, and spread of H3 AIVs. Sublineages of H3 AIVs have been detected worldwide, infecting various birds, at least 90 species in wild birds and poultry. Important areas for large-scale and local dissemination of H3 AIVs were identified, such as Alaska, Central Asia, and Chinese provinces. The H3 viruses have elevated the HA gene substitution rate after introduction from wild birds to domestic poultry, and even faster in domestic chickens. Our results implied an evolutionary mechanism of H3 AIV cross-species transmission, that viruses from wild birds to domestic poultry have accelerated substitution rate by shorter generation time and host selection. Novel chicken H3 viruses, especially H3N8 G25 viruses that have spilled over to humans, require high attention.

Keywords: Avian influenza virus; H3; Evolution; Spread; Phylogeography; Poultry; Wild bird

14. Minghui Li, Xiaojing Gao, Yunjing Zhang, Dongying Du, Wanbing Wang, Sumei Hong, Jialei Duan, Hui Tian, Lulu Wang, Zhuoyi Li, Wenqiang Pang, Kegong Tian, **Epidemiology, evolution, and biological characteristics of H3 avian influenza viruses isolated from chickens in China**, One Health, Volume 21, 2025, https://doi.org/10.1016/j.onehlt.2025.101153.

ABSTRACT

During an epidemiological investigation of avian influenza viruses (AIVs) in China, we isolated four H3 AIVs from chickens. To investigate the genetic relationships of these Chinese isolates with the globally circulating H3 viruses, we performed a detailed phylogenic analysis of the hemagglutination (HA) genes of 2613 representative H3 viruses available in the public source, and found that the HA genes of H3 viruses in China evolved from the Eurasian lineage and became established in domestic Anseriformes (primarily ducks). Bayesian phylodynamic analysis revealed that the Southern China (Guangdong and Guangxi provinces) served as a hub for the H3 virus diffusion to other parts of China, and the virus dissemination was potentially primarily driven by domestic ducks. Of note, the rate of H3N8/H3N3 virus detection had been increasing since 2021, and the main host of these H3 viruses appeared to have shifted from ducks to chickens, posing a potential pandemic threat within poultry populations. Here we showed that changes in amino acid substitutions located at antigenic sites around the receptor binding pocket of the HA protein, together with internal gene recombination of G57 H9N2 viruses, causing altered antigenicity and improved adaptability in chickens. The four H3 isolates in this study acquired multiple mutations for mammalian

adaption, and presented increased pathogenicity in mice. These findings emphasize that the continued evolution of these H3 viruses in poultry poses ongoing economic and pandemic threat, and highlight the need for continued surveillance of H3 viruses from poultry.

Keywords: H3 avian influenza viruses; Phylogenetic; Evolutionary dynamic; Antigenicity; Pathogenicity

15. Juliette Blais-Savoie, Emily Halajian, Kuganya Nirmalarajah, Andra Banete, Juan C. Corredor, Jonathon D. Kotwa, Yaejin Lee, Sugandha Raj, Shayan Sharif, Nicole Mideo, Samira Mubareka, **Examining the Threat of H5N1 Highly Pathogenic Avian Influenza to Human Health**, CHEST, 2025, https://doi.org/10.1016/j.chest.2025.10.030.

ABSTRACT

The clade 2.3.4.4b highly pathogenic avian influenza (HPAI) virus H5N1 is the etiologic agent for an ongoing panzootic with a rapidly increasing number of human infections. Although morbidity and mortality in humans with this clade seems to be limited to date, previous HPAI H5N1 viruses have been associated with mortality rates of approximately 50% in humans. Not all cases of clade 2.3.4.4b influenza A (H5N1) HPAI in humans have been associated with known exposure to infected animals. Therefore, clinicians must be aware of the changing viral ecology, human risk factors, and clinical presentations associated with H5N1 viruses to facilitate early case recognition and management of clade 2.3.4.4b A(H5N1) HPAI infection in humans.

Review Findings

Historic H5N1 presentations have involved multiorgan systemic disease, notably including severe neurological disease. Common symptoms associated with clade 2.3.4.4b A(H5N1) HPAI include conjunctivitis, fever, and upper respiratory tract infection. Exposure to infected dairy cattle is a novel risk factor.

Summary

The rapid global spread of clade 2.3.4.4b A(H5N1) viruses has been associated with severe disease and high mortality in many farmed animal species and wildlife. The composite picture of emerging risk to human health comprises an unprecedented number of mammalian infections, viral adaptations to mammalian hosts, severe neuroinvasive disease in naturally infected mammals, and spillover into novel species such as dairy cows with forward transmission to humans. Preparedness measures are crucial to mitigating significant human health impacts from this virus and must include a Canadian One Health Training Program in Emerging Zoonoses approach that promotes both animal and human health.

Keywords: acute respiratory infection; clade 2.3.4.4b; emerging pathogens; H5N1; H5Nx; One Health; viral zoonoses

16. Jiamin Fu, Ping Wang, Han Wu, Fan Yang, Linfang Cheng, Fumin Liu, Hangping Yao, Nanping Wu, Lihua Xu, Haibo Wu, **Development of a graphene oxide multilayer quantum dot-based immunochromatographic strip for the ultrasensitive detection of H7 subtype avian influenza viruses**, Poultry Science, Volume 104, Issue 4, 2025, https://doi.org/10.1016/j.psj.2025.104924.

ABSTRACT

Since March 2013, the H7N9 subtype of avian influenza virus (AIV) has become an important zoonotic infectious disease, garnering significant global attention because of its potential to affect human health. Establishing a rapid, effective, and sensitive method to detect H7 subtype AIVs is crucial for disease control. In this study, we developed a graphene oxide multilayer quantum dot-based immunochromatographic strip for the ultrasensitive detection of H7 subtype AIVs. The method demonstrated excellent sensitivity, with a limit of detection of 0.063 hemagglutinin units and 0.016 ng/ml for the hemagglutinin protein. The method exhibited remarkable specificity, with no reaction with other subtypes of influenza A virus andno cross-reactivity with other types of avian virus. Additionally, this method exhibited excellent reproducibility, with both inter-group and intra-group variations remaining below 10 %. Preliminary testing on avian clinical samples showed impressive consistency, underscoring the method's reliability. These initial results suggest that this detection approach has significant potential for widespread use in analyzing avian clinical samples, indicating substantial promise for its future application in various diagnostic settings.

Keywords: H7 subtype influenza virus; Immunochromatographic strip; Graphene oxide; Quantum dot; Ultrasensitive detection

17. Nan Zhang, Keji Quan, Mengqi Lin, Zijun Lu, Zhifan Li, Yiming Yang, Nuo Xu, Hui Yang, Jie Zhu, George Fei Zhang, Tao Qin, Sujuan Chen, Daxin Peng, Xiufan Liu, **Comprehensive evaluation of HA epitope modifications in H9N2 subtype avian influenza vaccines for broad cross-protection**, Journal of Integrative Agriculture, 2025, https://doi.org/10.1016/j.jia.2025.08.018.

ABSTRACT

The hemagglutinin (HA) protein of the H9N2 subtype avian influenza virus (AIV) undergoes frequent antigenic drift, which compromises the efficacy of existing inactivated vaccines. We have identified 12 key HA residues responsible for antigenic differences between the 2 major H9N2 antigenic groups; however, their role in eliciting broad cross-reactive immunity remains undefined. In this study, we systematically evaluated the impact of single- and multi-residue mutations in HA antigenic regions A, B1, B2, and E on viral antigenicity using antigenic cartography and monoclonal antibody profiling. 4 recombinant viruses—R118-A, R118-AE, R118-B1, and R118-AB1E—demonstrated broadened antigenic reactivity and were selected for further analysis. Among them, R118-A elicited immune sera with high hemagglutination inhibition and microneutralization titers against a diverse panel of H9N2 strains and exhibited broad antigenic coverage on antigenic cartography. In chicken challenge experiments,

immunization with R118-A conferred cross-protection against group 1 (B4.4+B4.6) and group 2 (B4.7) H9N2 viruses, underscoring the critical role of site A modifications in broadening vaccine protection. These findings offer theoretical support and practical strategies for the rational design of next-generation H9N2 vaccines with improved cross-protective efficacy.

Keywords: H9N2 avian influenza virus; Hemagglutinin; Monoclonal antibody profiling; Antigenic cartography; Broad-spectrum protection

18. Xinran Chu, Xuefeng Yin, Ye Tian, Wenjie Jiang, Zhehong Zhao, Jixiang Wang, Xudong Cao, Jianjun Sang, Quan Xie, Tuofan Li, Hongxia Shao, Aijian Qin, Jianqiang Ye, Zhimin Wan, **Characterizations of a novel triple-reassortant H3N3 avian influenza A virus isolated from chickens in China**, Poultry Science, Volume 104, Issue 12, 2025, https://doi.org/10.1016/j.psj.2025.106048.

ABSTRACT

The emergence of novel H3N8 and H10N3 avian influenza A viruses (IAVs) circulating in chicken flocks in China has raised significant concerns to their spillovers in humans and associated economic losses. In this study, we isolated a novel H3N3 strain from chickens exhibiting respiratory symptoms, designated as A/chicken/Shandong/118/2023(H3N3) (SD118). Genetic analyses revealed that SD118 was a triple-reassortment virus, possessing hemagglutinin (HA) gene originating from H3N8, neuraminidase (NA) gene originating from H10N3, and other six internal genes were from H9N2, each of which is currently circulating in chicken flocks in China. In vitro studies confirmed that SD118 could effectively replicate in mammalian cells (MDCK and A549 cells), and exhibited dual receptor-binding affinity for both avian-like (α-2, 3 sialic acid) and human-like (α-2, 6 sialic acid) receptors. Moreover, SD118 could replicated effectively not only in the lungs and nasal turbinates of the infected mouse with more than 10 % bodyweight loss, but also in the upper respiratory tract and intestines of the infected chickens with efficient transmission ability. Our findings highlight the ongoing evolution of H3 avian IAVs through reassortment with other subtypes in poultry and the enhanced surveillance is critical to monitor the genetic diversity and zoonotic potential of H3 viruses in chickens.

Keywords: H3N3; Triple-reassortment; Receptor binding; Replication; Pathogenicity

19. Sipei Zhang, Xiaolin Zhang, Boyu Liu, Chuanxiu Li, Xinyu Zhang, Xiang Li, Xinru Lv, Yi Li, Mengdan Fei, Qing An, Yang Xiu, Zhuoyan Li, Jingxin Liu, Linhong Xie, Hongliang Chai, **Eco-epidemiological mechanisms of avian influenza and Newcastle virus co-infection: Spatial convergence and compatibility**, Poultry Science, Volume 104, Issue 11, 2025, https://doi.org/10.1016/j.psj.2025.105779.

ABSTRACT

Understanding the mechanisms of viral co-infection in natural host populations is crucial for predicting pathogen dynamics and cross-species transmission risks. This study focuses on the

co-infection of avian influenza virus (AIV) and Newcastle disease virus (NDV) in wild ducks, combining field monitoring data, ecological niche modeling, and statistical analysis to reveal distribution patterns and driving factors. Organ samples collected from wild ducks in key migratory habitats revealed that AIV exhibits a broader ecological niche than NDV, with both viruses primarily replicating in the respiratory system. Ecological niche overlap modeling indicated a high degree of preference overlap between AIV and NDV in organ-specific microenvironments, suggesting that ecological compatibility is a prerequisite for co-infection. This study underscores the critical role of ecological and spatial compatibility in shaping viral co-infection patterns and provides a theoretical framework for understanding virus interactions in complex ecosystems.

Keywords: Avian influenza virus; Co-infection; Newcastle disease virus; Niche breadth; Niche overlap

20. Mana Esaki, Kosuke Okuya, Kaori Tokorozaki, Yuko Haraguchi, Jun Ito, Makoto Ozawa, **Surveillance of avian influenza viruses in the Izumi plain reveals the role of wild ducks in the introduction of H5N1 HPAIVs during the 2023/24 winter season**, Comparative Immunology, Microbiology and Infectious Diseases, Volume 123, 2025, https://doi.org/10.1016/j.cimid.2025.102389.

ABSTRACT

The Izumi plain, located in the southern part of Japan, serves as a major overwintering site for endangered crane species, including the hooded crane (Grus monacha) and the white-naped crane (Grus vipio). Since the 2012/13 winter season, continuous surveillance of avian influenza viruses (AIVs) in environmental water and wild birds has been conducted in this region. During the 2023/24 winter season, 45 isolates of H5N1 high pathogenicity AIVs (HPAIVs) and 24 isolates of low pathogenicity AIVs (LPAIVs) were obtained at different time points from crane roost water. Additionally, H5N1 HPAIVs were detected in four wild ducks in November 2023 and in eight cranes in December 2023. Phylogenetic analyses revealed that all H5N1 HPAIVs belonged to subclade G2d of clade 2.3.4.4b, with early winter isolates—particularly those from wild ducks and roost water—occupying more ancestral phylogenetic positions. These findings suggest that wild ducks likely introduced HPAIVs into the overwintering site. Genotype analysis based on the genetic constellations of all eight gene segments indicated the co-introduction of multiple HPAIV genotypes into the Izumi plain and suggested bidirectional gene segment exchange between HPAIVs and LPAIVs. Hemagglutination inhibition assays detected no H5 HA-specific antibodies in six overwintering cranes, implying a limited role for cranes in virus dissemination. Collectively, these findings underscore the importance of continued virological surveillance and genetic monitoring of AIVs at major overwintering sites, where close ecological interactions between wild ducks and cranes facilitate cross-species transmission.

Keywords: Avian influenza viruses; High pathogenicity avian influenza virus; Crane roost water; Cranes; Wild ducks

21. Lisa Bauer, Lonneke Leijten, Matteo Iervolino, Varun Chopra, Laura van Dijk, Mark Power, Willemijn Rijnink, Mark Pronk, Monique Spronken, Mathis Funk, Rory D de Vries, Mathilde Richard, Thijs Kuiken, Debby van Riel, **Attachment and replication of clade 2.3.4.4b influenza A (H5N1) viruses in human respiratory epithelium: an in-vitro study**, The Lancet Microbe, 2025, https://doi.org/10.1016/j.lanmic.2025.101230.

ABSTRACT

Background

Highly pathogenic avian influenza H5N1 viruses of the A/Goose/Guangdong/1/1996 lineage pose a global threat to wildlife, domestic animals, and humans. Cross-species transmission events to mammals, including humans, in the past 4 years highlight this threat. For influenza A viruses, crucial determinants of cross-species and intraspecies transmission to and among mammals include attachment to and replication in respiratory airway epithelial cells. Although these determinants have been studied for H5N1 viruses in the past, limited studies for clade 2.3.4.4b viruses exist. Therefore, the aim of this study was to determine the ability of recent clade 2.3.4.4b H5N1 viruses to attach to human respiratory tissues, to replicate in human airway epithelial cells and the associated immune response.

Methods

In this in-vitro study, we investigated three H5N1 clade 2.3.4.4b viruses (H5N1Gull2022, H5N1Polecat2022, and H5N1Bovine2024) in comparison with previously studied 2.1.3.2 H5N1 (H5N12005) and a seasonal H3N2 virus. First, we compared virus attachment patterns by virus histochemistry. Second, we investigated the infection and replication efficiency, and innate immune responses in infected human respiratory epithelium in vitro. Third, we measured polymerase complex activity using a minigenome assay.

Findings

Clade 2.3.4.4b viruses and H5N12005 virus differed by five amino acids located near the receptor binding site of the haemagglutinin. All clade 2.3.4.4b viruses attached more efficiently to cells of the human upper and lower respiratory tract compared with H5N12005 virus. All clade 2.3.4.4b viruses replicated in human nasal and tracheobronchial respiratory epithelium cultures. In the tracheobronchial respiratory epithelium cultures, H5N1Gull2022 virus replicated more efficiently than H5N12005 virus ($p=0{\cdot}0050$) and reached titres similar to H3N22003 virus. Polymerase complex activity of H5N1Gull2022 virus was not significantly different from that of H5N12005 and was significantly lower compared with H3N22003 virus ($p\leq0{\cdot}0001$). Infection with H5N1Gull2022 virus induced a broader antiviral immune response than H5N12005 virus.

Interpretation

Clade 2.3.4.4b H5N1 viruses have phenotypic characteristics that are different from a clade 2.1.3.2 H5N12005 virus. The ability of clade 2.3.4.4b viruses to attach to and replicate in

respiratory epithelium likely contributes to an increased risk for both human infection and virus adaptation to humans.

Funding

The EU, the Dutch Research Council, the Netherlands Organization for Health Research and Development, and the Dutch Ministries of Agriculture, Fisheries, Food Security and Nature, and Health, Welfare and Sport.

22. Hailiang Sun, Hanlin Liu, Jianfeng Zhang, Xiaoyun Qu, Zifeng Pang, Fengxiang Xu, Changrong Wu, Yinglin Jiang, Mang Shi, Quan Liu, Ming Liao, **Genome-scale evolution and phylodynamics of swine influenza A viruses in China: a genomic epidemiology study**, The Lancet Microbe, Volume 6, Issue 6, 2025, https://doi.org/10.1016/j.lanmic.2024.101020.

ABSTRACT

Background

Pigs are recognised as crucial intermediate hosts for the emergence of influenza viruses of pandemic potential. As the largest pork-producing nation, China hosts a complex ecosystem of swine influenza viruses (SIVs). We aimed to investigate the evolutionary processes, spatiotemporal dynamics, and biological characteristics of SIVs in China.

Methods

From Jan 15, 2016, to Dec 22, 2020, we collected nasal swabs from pigs at eight abattoirs and 16 swine farms in the Guangdong, Henan, and Shandong provinces of China, as part of SIV surveillance. SIVs were detected with RT-PCR. Positive samples underwent viral isolation and genome sequencing. We analysed evolution and spatiotemporal dynamics using the whole genomes of isolated SIVs, as well as genome sequences of SIV isolates from human infections worldwide retrieved from the Global Initiative on Sharing All Influenza Data and GenBank Flu databases up to April 28, 2024. Viral sequences without a sample collection area or date were excluded from the analysis. Viral receptor-binding properties and in-vitro replication of strains isolated in this study were evaluated with a solid-phase binding assay and various cell lines, including Madin-Darby canine kidney cells, porcine alveolar macrophages, primary porcine trachea epithelial cells, human bronchial epithelioid, and human lung adenocarcinoma epithelial (A549) cells. Viral replication and transmission studies were conducted in 33 guinea pigs and 13 pigs. Additionally, we collected serum samples from pig farm workers and members of the general public recruited by the Third Affiliated Hospital of Sun Yat-sen University between Feb 28 and May 11, 2023, to detect specific antibodies against Eurasian avian-like A(H1) and human-like A(H3N2) SIVs using the haemagglutination inhibition assay.

Findings

23 (1·3%) of 1818 nasal swabs collected in abattoirs had SIVs; 22 (0·9%) of 2375 swabs from swine farms had SIVs. Further viral isolation yielded 39 strains of SIV. We identified 534

A(H1N1), 69 A(H1N2), and 92 A(H3N2) SIVs, representing 20 genotypes within the Eurasian avian-like lineage, 14 within the classical swine A(H1) lineage, and 16 within the human-like A(H3N2) lineage. The introduction of the A(H1N1)pdm/09 virus significantly influenced the internal gene pool of SIVs, enhancing genotypic diversity in China. Notably, the Eurasian avian-like A(H1), classical swine A(H1), and human-like A(H3N2) lineages showed human-mediated spread over long distances between provinces, with the Eurasian avian-like A(H1) lineage showing the most prevalent spread pathways. Eurasian avian-like A(H1) SIVs showed a preference for binding to sialic acid α-2,6 glycan receptors, predominantly found in humans, resulting in an increased production of progeny viruses in human airway epithelial cells, as well as effective transmission and infectivity among guinea pigs and pigs. Among 54 eligible serum samples collected from pig farm workers (24 from slaughterhouses and 30 from swine farms), 23 (43%) were seropositive for Eurasian avian-like A(H1) SIVs and 46 (85%) for human-like A(H3N2) SIVs. Among 100 eligible samples from members of the general public, 14 (14%) were seropositive for Eurasian avian-like A(H1) SIVs and 85 (85%) for human-like A(H3N2) SIVs.

Interpretation

This study elucidates the evolutionary processes and spatiotemporal patterns of SIVs, highlighting potential risks to public health. These findings are crucial for informing public health interventions that aim to prevent future SIV epidemics in China and other countries worldwide.

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23. Jaemoo Kim, Jungho Kim, Suhyeon Heo, Chang-Hun Yeom, Bao Tuan Duong, Haan Woo Sung, Seon-Ju Yeo, Hyun Park, Haryoung Poo, Jihyun Yang, **A low pathogenic avian influenza A/Mallard/South Korea/KNU2019-34/2019 (H1N1) virus has the potential to increase the mammalian pathogenicity**, Virologica Sinica, Volume 40, Issue 1, 2025, https://doi.org/10.1016/j.virs.2024.12.005.

ABSTRACT

Influenza, a highly contagious respiratory infectious disease caused by an influenza virus, is a threat to public health worldwide. Avian influenza viruses (AIVs) have the potential to cause the next pandemic by crossing the species barrier through mutation of viral genome. Here, we investigated the pathogenicity of AIVs obtained from South Korea and Mongolia during 2018–2019 by measuring viral titers in the lungs and extrapulmonary organs of mouse models. In addition, we assessed the pathogenicity of AIVs in ferret models. Moreover, we compared the ability of viruses to replicate in mammalian cells, as well as the receptor-binding preferences of AIV isolates. Genetic analyses were finally performed to identify the genetic relationships and amino acid substitutions between viral proteins during mammalian adaptation. Of the 24 AIV isolates tested, A/Mallard/South Korea/KNU2019-34/2019 (KNU19-

34; H1N1) caused severe bodyweight loss and high mortality in mice. The virus replicated in the lungs, kidneys, and heart. Importantly, KNU19-34-infected ferrets showed high viral loads in both nasal washes and lungs. KNU19-34 replicated rapidly in A549 and bound preferentially to human like α2,6-linked sialic acids rather than to avian-like α2,3-linked sialic acids, similar to the pandemic A/California/04/2009 (H1N1) strain. Gene segments of KNU19-34 were distributed in Egypt and Asia lineages from 2015 to 2018, and the virus had several amino acid substitutions compared to H1N1 AIV isolates that were non-pathogenic in mice. Collectively, the data suggest that KNU19-34 has zoonotic potential and the possibility of new mutations responsible for mammalian adaptation.

Keywords: Avian influenza virus (AIV); H1N1; Zoonotic potential; Mutation; Receptor binding specificity; Ferret

24. Yan-He Wang, Jin-Jin Chen, Jun Ma, Jonathan E. Owen, Guo-Lin Wang, Lin-Jie Yu, Chun-Xi Shan, Yao Tian, Chen-Long Lv, Tao Wang, Yan Zhang, Sheng-Hong Lin, Xin-Jing Zhao, Sheng Zhang, Wang-Qian Wei, Yuan-Yuan Zhang, Tian Tang, Xin-Lou Li, Tao Jiang, Jing Li, Xiao-Ai Zhang, Feng Hong, Simon I. Hay, Yan-Song Sun, Wei Liu, Li-Qun Fang, **Early-warning signals and the role of H9N2 in the spillover of avian influenza viruses**, Med, Volume 6, Issue 7, 2025, https://doi.org/10.1016/j.medj.2025.100639.

ABSTRACT

Background

The spillover of avian influenza viruses (AIVs) presents a significant global public health threat, leading to unpredictable and recurring pandemics. Current pandemic assessment tools suffer from deficiencies in terms of timeliness, capability for automation, and ability to generate risk estimates for multiple subtypes in the absence of documented human cases.

Methods

To address these challenges, we created an integrated database encompassing global AIV-related data from 1981 to 2022. This database enabled us to estimate the rapid expansion of spatial range and host diversity for specific AIV subtypes, alongside their increasing prevalence in hosts that have close contact with humans. These factors were used as early-warning signals for potential AIV spillover. We analyzed spillover patterns of AIVs using machine learning models, spatial Durbin models, and phylogenetic analysis.

Findings

Our results indicate a high potential for future spillover by subtypes H3N1, H4N6, H5N2, H5N3, H6N2, and H11N9. Additionally, we identified a significant risk for re-emergence by subtypes H5N1, H5N6, H5N8, and H9N2. Furthermore, our analysis highlighted 12 key strains of H9N2 as internal genetic donors for human adaptation in AIVs, demonstrating the crucial role of H9N2 in facilitating AIV spillover.

Conclusions

These findings provide a foundation for rapidly identifying high-risk subtypes, thus optimizing resource allocation in vaccine manufacture. They also underscore the potential significance of reducing the prevalence of H9N2 as a complementary strategy to mitigate chances of AIV spillovers.

Funding

National Key Research and Development Program of China.

Keywords: avian influenza virus; H9N2; spillover; interspecies transmission; emerging infectious diseases; risk assessment; zoonosis; geo-epidemiology

25. Wanyue Zhang, Jérémie Prévost, Angela Sloan, Levi Tamming, Annabelle Pfeifle, Caroline Gravel, Sathya N. Thulasi Raman, Gary Van Domselaar, Michael J.W. Johnston, Lisheng Wang, Simon Sauve, Michael Rosu-Myles, Darwyn Kobasa, Anh Tran, Wangxue Chen, Xu Zhang, David Safronetz, Xuguang Li, Intranasal vaccine **induces broad and long-lasting immunity against the hemagglutinin stem of group 2 influenza A viruses, Antiviral Research**, Volume 243, 2025, https://doi.org/10.1016/j.antiviral.2025.106284.

ABSTRACT

Influenza A viruses are categorized into two phylogenetic groups (group 1 and group 2) based on the structure of their hemagglutinin (HA) protein. Within group 2, H3N2 poses a particular challenge due to its rapid evolution, limited vaccine efficacy, and association with more severe influenza seasons. Although T cell responses have been extensively studied in the context of vaccine-induced protection, HA stem (HA2)-specific T cell responses have been relatively understudied, especially those related to nasal immunity. To address this, we engineered an adenoviral vector vaccine (Ad-HA2) expressing a consensus hemagglutinin stem sequence, derived through bioinformatic analysis of all H3 strains. The vaccine conferred heterosubtypic protection against lethal challenges with either H3N2 or H7N9, both belonging to group 2 influenza A viruses, with protection lasting at least six months post-vaccination. Notably, the vaccine induced robust HA2-specific humoral and cell-mediated responses in the nasal-associated lymphoid tissue (NALT) of the upper respiratory tract, the first line of immune defense against inhaled pathogens. The vaccine also elicited significant levels of antibodies and T cell responses in the lower respiratory tract and pulmonary immune sites. Furthermore, circulating antibodies in the serum demonstrated effective antibody-dependent cellular cytotoxicity (ADCC) activity. Finally, using a peptide pool matrix screening approach combined with in silico verification, we identified an immunogenic C-terminus region of the HA2 consensus sequence that activated CD4+ and CD8+ T cells, which warrants further investigation. Collectively, these findings are informative for the design and evaluation of mucosal influenza vaccines targeting the hemagglutinin stem.

Keywords: Influenza; Hemagglutinin stem; Mucosal vaccine; T cell; H3N2; H5N1

26. Tangqi Wang, Ruiwen Han, Zhanyihao Hao, Xueting Cheng, Jia Li, Chengcheng Zhai, Junjia Guo, Donghong Wang, Yao Deng, Liang Zhang, Wenjie Tan, **Improved cross-protection and immunity against influenza A virus in mice using a novel mRNA vaccine with optimized design of RNA sequence**, Molecular Therapy Nucleic Acids, Volume 37, Issue 1, 2026, https://doi.org/10.1016/j.omtn.2025.102787.

ABSTRACT

Conventional influenza vaccines provide strain-specific immunity, and their production can be affected by egg-culture-adapted mutations. A universal influenza vaccine with conserved antigens and novel vaccine platforms is urgently required. To induce the immune responses toward more conserved epitopes, we generated a multi-antigen influenza mRNA vaccine, lipopolyplex (LPP)-HNH mRNA, coding headless hemagglutinin stem and neuraminidase with three different sequences (HNH-ORI, HNH-E1, or HNH-E2) and delivered by LPP. The immunogenicity and protective efficacy of these single-chain mRNA vaccines against influenza A viruses were evaluated in mice models. Mice exhibited sustained and robust antibody and cellular immune responses against all three LPP-HNH mRNA vaccines, indicating that these vaccines provided mice with broad protection against H1N1, H3N2, or H5N1 influenza viruses. HNH-E1 and HNH-E2 with optimized sequence showed higher protein expression, stronger specific CD4+ and CD8+ T cell responses, and more effective protection than those of HNH-ORI, owing to their lower minimum free energy and higher codon adaptation index. These results reveal that the LPP-HNH mRNA vaccine encoding conserved antigens with optimized sequences is a promising strategy for the development of universal influenza vaccines.

Keywords: MT: Delivery Strategies; influenza A virus; hemagglutinin stem; neuraminidase; broad-spectrum mRNA vaccine; optimized sequence

27. Malaika D. Argade, Varada Anirudhan, Sean P. Bradley, Łukasz Tomorowicz, Ryan Bott, Boopathi Sownthirarajan, Christian A. Zielinski, John P. Sloan, Dejan S. Nikolic, Arsen M. Gaisin, Terry W. Moore, Balaji Manicassamy, Norton P. Peet, Lijun Rong, Irina N. Gaisina, **Refinement of imidazo[2,1-a]pyrimidines in pursuit of potential drug candidates against group 2 influenza A viruses**, European Journal of Medicinal Chemistry, Volume 292, 2025, https://doi.org/10.1016/j.ejmech.2025.117679.

ABSTRACT

We discovered a series of imidazo[1,2-a]pyrimidines as potent, group 2 selective inhibitors of influenza A viruses (IAVs) that target the hemagglutinin-mediated viral entry process. Preliminary hit-to-lead optimization efforts afforded promising IAV inhibitors with improved activity against infectious H7N7 and H3N2 viruses. We now report a more comprehensive cycle of structure-activity relationship studies and optimization of metabolic stability, and overall druglike properties of this series of imidazo[1,2-a]pyrimidines, which allowed in the identification of two lead compounds that show promise as preclinical candidates. Compounds 10 and 12 inhibited pseudotyped H7N1 with EC50 values of 0.09 and 0.47 μM, respectively. They were among the most potent compounds in the viral replication assay

when tested against infectious H3N2 IAV, and they also demonstrated remarkable activity against avian influenza viruses; these data designated these imidazo[1,2-a]pyrimidines as potent broad-spectrum group 2 IAV inhibitors. Compounds 10 and 12 exhibit dissimilar but desirable drug metabolism and pharmacokinetics (DMPK) profiles, and therefore they offer different options for specific and effective patient treatment.

Keywords: Imidazopyrimidines; Dimroth rearrangement; Influenza A viruses; Anti-viral agent

28. Leon Schrell, David Scheibner, Antje Dickmanns, Kim M. Stegmann, Lukas Mathias Michaely, Annika Graaf-Rau, Philip Beer, Andrew Parker, Sandra Diederich, Anne Balkema-Buschmann, Matthias Dobbelstein, **Inhibitors of pyrimidine synthesis synergize with N4-hydroxycytidine to diminish influenza virus replication**, Antiviral Research, Volume 244, 2025, https://doi.org/10.1016/j.antiviral.2025.106286.

ABSTRACT

Influenza viruses remain a major threat to both human and animal health, with seasonal outbreaks and the risk of pandemics caused by reassortant strains. Antiviral drugs are needed as a complement to vaccines, but resistance often limits their long-term efficacy. N4-hydroxycytidine (NHC), the active form of Molnupiravir, shows potent activity against influenza A viruses (IAVs) in both cell cultures and animal models, with minimal resistance observed. Building on prior work in SARS-CoV-2, we investigated whether inhibiting pyrimidine biosynthesis could enhance NHC's antiviral activity against IAVs. The combination of NHC with inhibitors of dihydroorotate dehydrogenase (DHODH) or cytidine triphosphate synthases (CTPS1/2) showed strong synergy. This was evident through reduced cytopathic effects, decreased viral RNA and protein, and a marked absence of infectious virus particles. This synergy was consistent across multiple IAV subtypes, including H1N1, H1N2, H3N2, and H5N1. This synergistic effect was reversed by exogenously supplemented pyrimidine nucleosides, confirming nucleotide depletion as a key mechanism. However, some avian IAVs were less sensitive to the treatment in mammalian cells. The PB2-K627E mutation, affecting the interaction with host factor ANP32, modulated NHC efficacy, implicating viral adaptation in drug responsiveness. In a ferret model of H5N1 infection, NHC combined with the CTPS inhibitor STP938 reduced clinical symptoms and lung pathology, with NHC mostly driving antiviral activity and STP938 contributing to disease mitigation. These findings indicate that combining NHC with pyrimidine biosynthesis inhibitors enhances antiviral efficacy against IAVs, especially in rapidly replicating viruses, and may broaden the utility of nucleoside analogues in influenza therapy.

Keywords: IAV; N4-hydroxycytidine; Molnupiravir; Dihydroorotate dehydrogenase (DHODH); Cytidine triphosphate synthase (CTPS); Pyrimidine analogues; RNA polymerase; Host adaption; ANP32

29. Anika John, Seju Kang, Lara Fuhrmann, Ivan Topolsky, Christopher Kent, Joshua Quick, Tanja Stadler, Timothy R. Julian, Niko Beerenwinkel, **Characterizing influenza A virus lineages and clinically relevant mutations through high-coverage wastewater sequencing**, Water Research, Volume 287, Part B, 2025, https://doi.org/10.1016/j.watres.2025.124453.

ABSTRACT

Influenza A virus poses significant public health challenges, causing seasonal outbreaks and pandemics. Its rapid evolution motivates continuous monitoring of circulating influenza genomes to inform vaccine and antiviral development. Wastewater-based surveillance offers an unbiased, cost-effective approach for genomic surveillance. We developed a novel tiling amplicon primer panel that covers diversity of influenza A virus, targeting segments of the surface proteins HA, NA, and M of subtypes H1N1 and H3N2. Using this panel, we sequenced nucleic acid extracts from 59 Swiss wastewater samples collected at four locations during the 2022/2023 and 2023/2024 winter seasons. We found that wastewater-based abundance estimates of the dominant H1N1 clades correlated with clinical-based estimates in the 2023/2024 season. Furthermore, wastewater-based sequencing revealed mutations in vaccine and drug target sites, consistent with clinical data. Overall, we demonstrate the effectiveness of wastewater-based genomic surveillance of influenza A, including lineage identification and mutation tracking to inform vaccine and antiviral strategies.

Keywords: Wastewater-based epidemiology; Influenza A; Genomic surveillance; Public health

30. Yingjun Li, Jianfang Zhang, Fengming He, Cuiting Cao, Yangqing Zhan, Nanshan Zhong, Zifeng Yang**, Anti-influenza drugs targeting trimeric RNA polymerase complex: From development to clinics**, Acta Pharmaceutica Sinica B, 2025, https://doi.org/10.1016/j.apsb.2025.11.033.

ABSTRACT

The rapid evolution of influenza viruses, driven by high mutation rates and cross-species transmission, underscores the importance of discovering antivirals with novel mechanisms of action and distinct resistance profiles. The influenza virus RNA polymerase, a highly conserved heterotrimeric complex, comprises polymerase basic protein 1 (PB1), polymerase basic protein 2 (PB2), and polymerase acidic protein (PA) in influenza A and B viruses, or polymerase 3 protein (P3) in influenza C and D viruses. This complex is essential for viral genome replication and transcription, rendering it a critical target for antiviral intervention. Over the past two decades, research on influenza polymerase (FluPol) has advanced from fundamental studies to drug development and clinical application. At the time of this review's publication, five FluPol-targeting drugs have received regulatory approval: the PA inhibitors baloxavir marboxil, suraxavir marboxil, and seloxavir marboxil; the PB1 inhibitor favipiravir; and the PB2 inhibitor onradivir, with several additional candidates progressing to clinical research. This review summarizes the structure and function of influenza polymerase and the mechanisms

of action of different inhibitors, highlighting the discovery and clinical effectiveness of the newly approved FluPol-targeting drugs. It addresses the potential of FluPol inhibitors against highly pathogenic avian influenza and the challenges posed by resistance mutations.

Keywords: Influenza; RNA polymerase; Antiviral drug; Polymerase basic protein 1; Polymerase basic protein 2; Polymerase acidic protein; Approved drug; Replication and transcription; Drug resistance; Structure-based drug design

31. Jingze Liu, Xu Zhang, Shicheng Li, Xiao Ding, **Genome-wide identification of characteristic nucleotide fragments for surveillance and subtype typing of influenza A viruses**, Decoding Infection and Transmission, Volume 3, 2025, https://doi.org/10.1016/j.dcit.2025.100056.

ABSTRACT

Influenza A viruses, members of the Orthomyxoviridae family, are major causative agents of past flu pandemics and can infect a wide range of hosts depending on their hemagglutinin (HA) and neuraminidase (NA) gene combinations. This study aimed to identify genome-wide characteristic nucleotide fragments for rapid detection and subtype typing of influenza A viruses from large-scale genomic data.

Methods

Complete influenza genome sequences were analyzed to identify candidate characteristic fragments specific to influenza A viruses. The fragments were evaluated based on conservation probability, coverage, and specificity across different viral species, subtypes, and hosts. High-coverage fragments were selected for further analysis. Multiplex PCR primers were then designed based on the selected fragments, and their predictive performance was assessed via in silico PCR.

Results

Characteristic fragments from the M gene (20–40 bp) distinguished influenza A viruses with >92 % coverage and >99 % specificity. Forty-four fragments from the HA gene were identified across 26 subtypes, indicating the HA gene's utility for subtype differentiation. Regarding host specificity, canine-derived strains contained unique 20–40 bp fragments, the avian-specific fragment was 20 bp, and no such fragments were detected in other hosts. The designed primers achieved >98 % predicted accuracy for universal detection (M gene) and for H1N1- and H3N2-specific subtypes.

Conclusion

Genome-wide screening of influenza A virus sequences identified highly conserved and subtype-specific nucleotide fragments that enable rapid detection and precise subtyping. These findings provide a valuable resource for molecular surveillance and diagnostic assay development.

Keywords: Influenza A virus; Characteristic fragment; Genome-wide; Surveillance; Subtype

32. Mélissa Bessonne, Jessica Morel, Quentin Nevers, Julie Groutsch, Agathe Urvoas, Marie Valerio-Lepiniec, Thibaut Crépin, Philippe Minard, Bernard Delmas, **Inhibition of influenza virus replication by artificial proteins (αReps) targeting its RNA-polymerase**, Antiviral Research, Volume 244, 2025, https://doi.org/10.1016/j.antiviral.2025.106300.

ABSTRACT

Seasonal epidemics and pandemics caused by influenza A viruses still represent a main public health burden in the world. Influenza viruses replicate and transcribe their genome in the nucleus of the infected cells, two functions that are supported by the viral RNA-dependent RNA-polymerase (FluPol) through extensive structural rearrangements and differential interactions with host cell factors. To get insights into its functioning, we screened a phage-display library of biosynthetic proteins (named αReps and built on a rigid alpha-helicoidal HEAT-like scaffold) against the structurally invariant FluPol core and several flexibly-linked domains of the FluPol PB2 subunit. Several αReps specific of the cap binding domain [CBD], the 627-domain and the NLS domain of PB2 displayed FluPol inhibitory and virus neutralization activities when transiently expressed in the cytosol. Furthermore, intracellular ectopic inducible expression of the αReps C3 and F3 (specific of the CBD and the 627-domain, respectively) in influenza virus permissive cells blocked multiplication of viruses representative of the H1N1, H3N2 and H7N1 subtypes, even when induced at late times post-infection. Bispecific αReps constructs (C3-F3 and F3-C3) display a higher FluPol inhibitory activity than their monomeric counterparts. These results suggest that interfering with FluPol structural rearrangements may represent a promising strategy to block virus multiplication and to design new types of antivirals such as dual binders targeting distant sites on FluPol. Furthermore, we found that the 627-domain constitutes a new possible target for engineering influenza antivirals.

Keywords: Influenza virus; RNA-Dependent RNA-Polymerase; Artificial protein; Nanobinder; PB2 subunit; Intracellular neutralization

33. Weijie Chen, Shuiping Lu, Haiyan Xiong, Zhiyu Xiang, Yuxi Wang, Jingjing Hu, Yue Pan, Yanjiao Li, Qile Gao, Qi Chen, Siru Hu, Weibing Wang, Chenglong Xiong, **Gene flow and its sporadic spillover: H10 and N5 avian influenza viruses from wild birds and the H10N5 human cases in China**, Virologica Sinica, Volume 40, Issue 1, 2025, https://doi.org/10.1016/j.virs.2024.12.002.

ABSTRACT

On January 30, 2024, China announced the first human case of H10N5 influenza infection. Prior to this, human cases of H10N7 and H10N8 had been reported. It is now appropriate to re-examine the evolution and future epidemiological trends of the H10 and N5 subtypes of avian influenza viruses (AIVs). In this study, we analyzed the reassortment characteristics of the first human-derived H10N5 AIV (A/Zhejiang/ZJU01/2023), as well as the evolutionary

dynamics of the wild bird-derived H10 and N5 subtypes of AIVs over the past decade. Our findings indicate that the human-derived H10N5 AIV exhibited low pathogenicity. A/bean_goose/Korea/KNU-10/2022(H10N7) and A/mallard/Novosibirsk_region/962k/2018(H12N5) were identified as the potential reassortment parents. The virus has existed since 2022 and several isolations have been reported in Bangladesh. Phylogenetic analysis showed that H10Ny and HxN5 AIVs in China are clustered differently based on the East Asian-Australian (eastern) and Central Asian-Indian (western) migratory flyways. The H10Ny and HxN5 AIV reassortant strains may cause human infections through accidental spillover. It is possible that another center of AIV evolution, mutation, and reassortment may be developing along the migratory flyways in northeastern Asia, distinct from Europe, the Americas, and China's Yangtze River Delta and Pearl River Delta, which should be closely monitored to ensure the safety of the public.

Keywords: Avian influenza virus (AIV); H10N5; Reassortment; Evolution; Migration flyway

34. Martha P. Montgomery, Prabda Praphasiri, Darunee Ditsungnoen, Pasakorn Akarasewi, Malinee Chittaganpitch, Pilaipan Puthavathana, Khanchit Limpakarnjanarat, Ponthip Wirachwong, Tawee Chotpitayasunondh, Narumol Sawanpanyalert, Chaninan Sonthichai, William W. Davis, Sonja J. Olsen, Supamit Chunsuttiwat, **Influenza surveillance and vaccine policy in Thailand—a historical perspective**, The Lancet Regional Health - Southeast Asia, Volume 41, 2025, https://doi.org/10.1016/j.lansea.2025.100663.

ABSTRACT

Prior to 2000, influenza burden in Thailand and other low- and middle-income countries was underappreciated, and influenza vaccination was uncommon. For the last two decades, Thailand Ministry of Public Health (MOPH) and U.S. Centers for Disease Control and Prevention have collaborated to understand influenza burden and the costs and benefits of influenza vaccination in Thailand. Built on a long-standing national disease notification system, Thailand MOPH established robust surveillance platforms for pneumonia and influenza, which provided insights into seasonality, disease incidence, and populations at risk for severe disease. In 2004, human cases of avian influenza brought attention to influenza's pandemic potential. Concern for an influenza pandemic combined with evidence of the cost effectiveness of influenza vaccination accelerated vaccine policy. Surveillance and vaccination policy were leveraged for and strengthened by the 2009 influenza H1N1 and COVID-19 pandemics. This personal view documents Thailand's experience in developing influenza surveillance and influenza vaccination policy.

Keywords: Influenza; Vaccination; Thailand; Surveillance

35. Mengying Liu, Xuesheng Wu, Martijn D.B. van de Garde, Yoshiki Narimatsu, Frank J.M. van Kuppeveld, Henrik Clausen, Cornelis A.M. de Haan, Erik de Vries, **Cell-based sialoglycan arrays for directly comparing influenza A virus receptor requirements**

for binding and infection, iScience, Volume 28, Issue 6, 2025, https://doi.org/10.1016/j.isci.2025.112549.

ABSTRACT

Influenza A viruses multivalently engages sialoglycan attachment factors. Synthetic glycan arrays provide meticulous insight into primary binding specificity but do not capture dynamic post-binding virus-receptor interactions leading to cell entry. Establishing an HEK293 cell-based array of genetically dissected sialoglycan assemblies enabled screening of the complete interaction cascade from binding to infection, at physiologically relevant low virus doses. Screening forty years of H3N2 receptor binding evolution showed that besides N-glycans, deemed as principal receptors for primary attachment, specific O-glycans or glycosphingolipids independently supported all steps from primary binding to entry. For all three glycoconjugate classes, receptor preferences gradually evolved toward utilization of human-type α2-6-linked sialic acid receptors, followed by regaining use of avian-type α2-3-linked receptors after 1995. The screen identified a lack of quantitative correlation between binding and infection efficiency, suggesting specific receptor requirements beyond attachment. Virus-glycan interactions and other sialoglycan-dependent interactions with cells can be functionally analyzed using this system.

Keywords: Biological sciences; Microbiology; Natural sciences; Virology

36. Disha Bhavsar, André Nicolás León, Wei-Li Hsu, Eduard Puente-Massaguer, James A. Ferguson, Julianna Han, Patrick Wilson, Andrew B. Ward, Florian Krammer**, Structural and functional characterization of the antigenicity of influenza A virus hemagglutinin subtype H15**, Cell Reports, Volume 45, Issue 1, 2026, https://doi.org/10.1016/j.celrep.2025.116773.

ABSTRACT

Avian H15 influenza viruses are closely related to H7 viruses, but only 22 H15 sequences have been reported since 1987, suggesting both rarity and minimal antigenic variation. Here, we characterized a panel of mouse monoclonal antibodies (mAbs) raised against the A/wedge-tailed shearwater/Western Australia/2576/1979 ancestral strain, and a human mAb isolated from an H7N9 vaccinee. We found differences in binding and neutralization profiles against the ancestral strain and drifted strains of H15 isolated after 2008. mAbs exhibiting hemagglutination inhibition activity against the ancestral strain do not show binding to drifted strains, hinting at antigenic differences near the receptor binding site. We show that the mAbs protect in vivo and elucidate mAb-antigen interactions using negative stain and cryo-electron microscopy. The characterization of H15 antigenicity and the mechanisms of antibody-mediated neutralization expands our knowledge of this sparsely sampled avian influenza virus subtype and informs our understanding of immune pressures on viral surface glycoproteins.

Keywords: avian influenza; structural virology; hemagglutinin; H15; antigenic drift

37. Ane Marie Anderson, Elias Tjarnhage, Daniëla Maria Hinke, Ranveig Braathen, Gunnveig Grodeland, Bjarne Bogen, **DNA vaccines targeting hemagglutinin from 18 subtypes of influenza A virus to antigen presenting cells confer broad protection**, Molecular Therapy Nucleic Acids, 2025, https://doi.org/10.1016/j.omtn.2025.102814.

ABSTRACT

Novel vaccines that confer broad protection against influenza A viruses (IAV) are urgently needed. Hemagglutinin (HA) is the major influenza antigen targeted by protective immune responses. We have here developed a DNA vaccine that simultaneously presents HA from 18 subtypes of IAV to the immune system. The vaccine consists of a DNA plasmid mixture that encodes a variety of dimeric vaccine proteins. Each dimer expresses two different HA, as well as a targeting moiety directing the vaccine protein to antigen presenting cells (APC). When the vaccine proteins were targeted towards chemokine receptors 1, 3 and 5 (CCR1/3/5) on APC by means of MIP1α (CCL3), vaccinated mice were broadly protected against infection with H1N1, H3N2, H5N1 and H7N1 influenza viruses. Furthermore, antibody mediated protection against H1N1 was maintained when the H1 antigen was removed from the plasmid mixture, indicating that the diversity of HAs in the mixture promoted formation of antibodies specific for shared, conservative epitopes. The results may guide development of a broadly protective influenza A vaccine for humans.

38. Minami Komami, James G. Komu, Yuki Ishiguro, Motoki Sasaki, Sachiko Matsuda, Dulamjav Jamsransuren, Vuong Nghia Bui, Yohei Watanabe, Kunitoshi Imai, Haruko Ogawa, Yohei Takeda, **Detection of antibodies against H5 subtype highly pathogenic avian influenza viruses in multiple raccoons in Tokachi District, Hokkaido, Japan, from 2022 to 2023**, Virus Research, Volume 351, 2025, https://doi.org/10.1016/j.virusres.2024.199515.

ABSTRACT

In recent years, infection cases of H5 subtype highly pathogenic avian influenza viruses (HPAIVs) in wild mammals have increased globally. To obtain recent epidemiological information regarding influenza A virus (IAV) infection in raccoons (Procyon lotor), the prevalence of anti-IAV antibodies in sera was analyzed among raccoons captured in Tokachi District, Hokkaido, Japan, from 2019 to 2023. Screening of serum samples using enzyme-linked immunosorbent assay and agar gel precipitation test detected anti-IAV antibodies in 5 of 114 (4.4 %) raccoons. All positive sera were from raccoons captured from 2022 to 2023. The hemagglutination inhibition test revealed that all five serum samples contained anti-H5 subtype HPAIV antibodies, and one also contained anti-H1 subtype antibodies. The neuraminidase inhibition test revealed that all five sera contained anti-N1 subtype antibodies, and one also contained anti-N8 subtype antibodies. In the virus neutralization test, these five sera showed stronger neutralization activity against the H5 subtype clade 2.3.4.4b HPAIV strain recently circulating worldwide compared to the old H5 HPAIV strain isolated in Japan

in 2007. These findings suggested that raccoons could be involved in the circulation of H5 HPAIVs in nature.

Keywords: H5 subtype highly pathogenic avian influenza virus; Clade 2.3.4.4b; Raccoon; Seroprevalence

39. Mansoor Ashraf, Alicia N. Stein, John Youhanna, Steven Rockman, Meagan McMahon, Ian McGovern, Sankarasubramanian Rajaram, Matthew S. Miller, **The impact of egg adaptation and immune imprinting on influenza vaccine effectiveness**, Vaccine, Volume 62, 2025,

ABSTRACT

Most influenza vaccines are produced in hens' eggs and may undergo ‘egg adaptation’, whereby mutations within the haemagglutinin protein that result in adaptation to the avian cells undergo positive selection. It is well established that egg adaptation can impact antigenicity and vaccine effectiveness (VE) by causing mismatches between the vaccine virus and circulating viruses. However, few studies have investigated the potentially long-lasting impact of childhood vaccination with an egg-adapted vaccine on the immunological memory. Prior exposure history shapes subsequent immune responses, such that memory responses to previously encountered antigens trigger stronger immune responses than those elicited by de novo antigen exposure. This phenomenon is called immune imprinting, when referring specifically to the impact of the first lifetime exposure, and antigenic seniority, when referring to exposures after the first, which also shape an individual's antibody repertoire according to how early and how often they are encountered. Crucially, if an individual's first influenza exposure is via an egg-adapted vaccine, this imprinting event could adversely affect antibody responses to circulating viruses in future seasons, reducing the benefit of influenza vaccination. Using alternative types of vaccines that avoid egg adaptation is particularly important now that the World Health Organization (WHO) recommend immunising children aged ≥6 months against influenza. In this review, we cover the historical frequency and nature of egg adaptations and the impact of egg adaptation, immune imprinting and antigenic seniority on the clinical effectiveness of seasonal influenza vaccinations. We discuss the impact of interactions between egg adaptation and immune imprinting, examine how egg-adapted vaccines can lead to suboptimal imprinting and potentially reduce VE throughout an individual's lifetime, and identify how we can address this issue in future.

Keywords: Egg-adapted mutations; Immune imprinting; Antigenic seniority; Cell-based vaccines; Vaccine effectiveness; Influenza

40. Won Suk Choi, Jacob Lee, Cecilia Ottaviano, Sandrine Samson, Lin Peng, Sooyoun Shin, Sunho Choe, Woo Joo Kim, **Immunogenicity and safety of quadrivalent recombinant influenza vaccine in Korean adults: Phase III, randomized study**, Vaccine, Volume 62, 2025, https://doi.org/10.1016/j.vaccine.2025.127521.

ABSTRACT

Quadrivalent recombinant influenza vaccine (RIV4) is indicated for active immunization against influenza virus in adults (≥18 years) in countries where it is currently registered. This Phase III, parallel, randomized, modified double-blind, active controlled, multi-center study was designed to compare the immunogenicity and safety of single dose intramuscular RIV4 with a locally–licensed quadrivalent–inactivated influenza vaccine (IIV4, Fluarix® quadrivalent) in participants aged ≥18 years during 2021–22 Northern Hemisphere influenza season at three tertiary care centers in South Korea. Participants were randomized (1:1) to receive single dose intramuscular RIV4 or IIV4. All participants were centrally assigned to randomized study intervention using an interactive response technology (IRT). Participants, investigators, and the staff in-charge of the sampling, safety assessment and immunogenicity assays were blinded. The overall mean age of 300 participants who completed the study was 46.1 years. The post-vaccination geometric mean fold-rise of HAI antibodies were 32.0, 13.2, 6.1, and 5.3 in RIV4 group, and 22.3, 8.0, 4.7, and 3.4 in IIV4 group for the A/H1N1, A/H3N2, B/Victoria lineage, and B/Yamagata lineage strains, respectively. Seroconversion in RIV4 was comparable or higher than IIV4. Neither immediate unsolicited AEs were reported within 30 min of vaccination nor SAEs, AESIs nor deaths were reported during the 6-month follow-up. Clinical Trial Registration:ClinicalTrials.gov number – NCT05144945.

Keywords: Recombinant influenza vaccine; Immunogenicity; Safety; Clinical trial; Korean adults; Influenza vaccination

41. Saira Hussain, Adam Meijer, Elena A. Govorkova, Clyde Dapat, Larisa V. Gubareva, Ian G. Barr, Sook Kwan Brown, Rod S. Daniels, Seiichiro Fujisaki, Monica Galiano, Weijuan Huang, Rebecca J. Kondor, Angie Lackenby, Nicola Lewis, Janice Lo, Ha T. Nguyen, Mira C. Patel, Dmitriy Pereyaslov, Aine Rattigan, Magdi Samaan, Dayan Wang, Richard J. Webby, Hui-Ling Yen, Wenqing Zhang, Emi Takashita, **Global update on the susceptibilities of influenza viruses to neuraminidase inhibitors and the cap-dependent endonuclease inhibitor baloxavir, 2020–2023**, Antiviral Research, Volume 241, 2025, https://doi.org/10.1016/j.antiviral.2025.106217.

ABSTRACT

Antiviral susceptibility of influenza viruses is monitored by the World Health Organization Global Influenza Surveillance and Response System. This study describes a global analysis of the susceptibility of influenza viruses to neuraminidase (NA) inhibitors (NAIs, oseltamivir, zanamivir, peramivir, laninamivir) and the cap-dependent endonuclease inhibitor (CENI, baloxavir) for three periods (May to May for 2020–2021, 2021–2022 and 2022–2023). In particular, global influenza activity declined significantly in 2020–2021 and 2021–2022 when compared to the pre-pandemic period of COVID-19. Combined phenotypic and NA sequence-based analysis revealed that the global frequency of seasonal influenza viruses with reduced or highly reduced inhibition (RI/HRI) by NAIs remained low, 0.09% (2/2224), 0.12% (27/23465) and 0.23% (124/53917) for 2020–2021, 2021–2022 and 2022–2023, respectively. As in previous years, NA-H275Y in A(H1N1)pdm09 viruses was the most frequent substitution

causing HRI by oseltamivir and peramivir. Sequence-based analysis of polymerase acidic (PA) protein supplemented with phenotypic testing revealed low global frequencies of seasonal influenza viruses with reduced susceptibility (RS) to baloxavir, 0.07% (1/1376), 0.05% (9/18380) and 0.12% (48/39945) for 2020–2021, 2021–2022 and 2022–2023, respectively; commonly associated substitutions were PA-I38T/M/L. In Japan, the rate was 3.3% (16/488) during 2022–2023, with 11 A(H3N2) viruses having PA-I38T/M substitutions. For zoonotic viruses, 2.7% (3/111) contained substitutions, one each NA-H275Y, NA-S247N and NA-N295S, associated with RI/HRI NAI phenotypes, and none contained PA substitutions associated with RS to baloxavir. In conclusion, the great majority of seasonal and zoonotic influenza viruses remained susceptible to NAIs and CENI baloxavir.

Keywords: Influenza; Antiviral; Neuraminidase inhibitor; Polymerase inhibitor; Baloxavir; Reduced susceptibility

42. Jing Wang, Saisai Guo, Jianyuan Zhao, Tingting Sun, Yilu Ye, Rui Zhou, Tao Deng, Xiaoyu Li, Jianwei Wang, Shan Cen**, Host lncRNA assists the nuclear import of influenza A virus protein PB2 in a species-specific manner**, Journal of Infection, Volume 91, Issue 2, 2025, https://doi.org/10.1016/j.jinf.2025.106540.

ABSTRACT

Long noncoding RNAs (lncRNAs) have been reported to modulate immune responses to viral infections. However, it remains largely unexplored how viruses exploit host lncRNAs to promote viral replication. Here, we found that an lncRNA, called lnc-ALOX12, is upregulated specifically in cells infected by influenza A virus (IAV). Lnc-ALOX12 promotes IAV infection through associating with IAV RNA polymerase subunit PB2, sustaining the interaction between PB2 and importin-α/β, thus warranting PB2 nuclear import and efficient viral RNA synthesis. Importantly, avian influenza A virus needs to mutate its PB2 protein in order to hijack human lnc-ALOX12 for efficient viral RNA synthesis in mammal cells. Therefore, our data support the dependence of IAV replication on host lncRNAs. This dependence is species-specific and acts as a barrier to cross-species transmission of avian influenza viruses.

Keywords: LncRNA; Influenza A virus; PB2; Nuclear import; Cross-species transmission

43. Mengchan Hao, Jiaying Wu, Lina Ji, Yubo Zhao, Shunyuan Zhang, Yiwei Guan, Liangyu Li, Wenxue Yang, Yuan Zhang, Jianjun Chen, **Pathogenicity, transmissibility, and receptor binding of a human-isolated influenza A (H10N5) virus**, mBio, Volume 16, Issue 8, 2025, https://doi.org/10.1128/mbio.00731-25.

ABSTRACT

Recently, human infections with H10 influenza viruses, including H10N8 and H10N3, have been reported. In January 2024, a case of H10N5 and H3N2 co-infection was reported in Zhejiang Province, China, which is the first human infection with H10N5 avian influenza virus (AIV) globally. Almost simultaneously, we isolated a wild bird-derived H10N5 strain similar to

the human H10N5 strain. To assess the public health risk, it is necessary to understand the zoonotic characteristics of these novel H10N5 viruses. Here, we evaluated the biological characteristics of human H10N5, wild bird H10N5, as well as poultry H10N8 in vitro and in vivo. We demonstrate that the novel H10N5 isolates infected and replicated effectively in human lung epithelial cells. They infected BALB/c mice without adaptation, which exhibited robust pathogenicity and caused mouse death. In guinea pig transmission experiments, the H10N5 strain spread through neither direct contact nor airborne exposure, whereas H10N8 transmitted effectively. Additionally, H10N5 exhibited dual receptor-binding characteristics with a stronger preference for avian receptors. The current public health risk of H10N5 is low. However, the occasional spillover infections of H10 AIV into humans and dual receptor-binding characteristics suggest a potential risk of cross-species transmission.

IMPORTANCE

In 2024, the H10N5 AIV was first reported to infect humans. Concurrently, we isolated a strain of H10N5 from wild birds that was highly similar to the human H10N5 strain. However, the zoonotic potential and the associated public health risks of the H10N5 virus remain unclear. In this study, we systematically evaluated the replication characteristics of human H10N5, wild bird H10N5, and poultry H10N8 in human lung epithelial cells, the virulence in mice, the transmission capabilities in guinea pigs, and the receptor-binding properties. Our results demonstrate that these novel H10N5 viruses have not yet acquired the ability to transmit in guinea pigs, but they possess the potential to infect mammals. These findings provide timely insights and warnings for the development of public health prevention strategies.

In 2024, the H10N5 AIV was first reported to infect humans. Concurrently, we isolated a strain of H10N5 from wild birds that was highly similar to the human H10N5 strain. However, the zoonotic potential and the associated public health risks of the H10N5 virus remain unclear. In this study, we systematically evaluated the replication characteristics of human H10N5, wild bird H10N5, and poultry H10N8 in human lung epithelial cells, the virulence in mice, the transmission capabilities in guinea pigs, and the receptor-binding properties. Our results demonstrate that these novel H10N5 viruses have not yet acquired the ability to transmit in guinea pigs, but they possess the potential to infect mammals. These findings provide timely insights and warnings for the development of public health prevention strategies.

Keywords: novel H10N5; replication; pathogenicity; transmissibility; receptor binding

44. Ruonan Liang, Francesca Peccati, Niels L.D. Ponse, Elif Uslu, Annelies J.H. de Rooij, Alvin X. Han, Geert-Jan Boons, Luca Unione, Robert P. de Vries, **Epistasis in the receptor-binding domain of contemporary H3N2 viruses that reverted to bind sialylated di-LacNAc repeats**, Cell Reports, Volume 44, Issue 8, 2025, https://doi.org/10.1016/j.celrep.2025.116007.

ABSTRACT

Since their introduction into humans, H3N2 influenza A viruses have evolved continuously to escape immunity through antigenic drift, driven by mutations in and around the receptor-

binding site. Recently, these changes resulted in viruses that recognize elongated glycans, which are less abundant in the human respiratory tract, complicating vaccine strain propagation. This study employed ELISA, glycan arrays, tissue staining, flow cytometry, and hemagglutinin (HA) assays to demonstrate the molecular determinants of recent H3N2 viruses that regained recognition of shorter glycans. Mutations Y159N/T160I in contemporary strains replace Y159/T160, weakening receptor binding. However, this is compensated by Y195F in the 190-helix. These findings highlight epistasis across critical residues in the HA receptor-binding site, including the 130-loop, 150-loop, and 190-helix. Interestingly, a positive correlation exists between binding to an asymmetrical N-glycan and binding to human and ferret respiratory tract tissues. These results elucidate the epistatic nature of receptor-binding specificity during influenza A virus H3N2 evolution.

Keywords: H3N2; influenza; sialic acid; N-glycan; receptor binding; hemagglutinin; epistasis

45. Aya Fujikane, Ryosuke Fujikane, Yusuke Sechi, Akinori Nishi, Yoshizumi Ishino, Tetsuya Hiyoshi, Atsuhiko Sakamoto, Shigeki Nabeshima**, Multiple antiviral mechanisms of Ephedrae Herba and Cinnamomi Cortex against influenza: inhibition of entry and replication**, Microbiology Spectrum, Volume 13, Issue 6, 2025, https://doi.org/10.1128/spectrum.00371-25.

ABSTRACT

Maoto, a traditional herbal medicine widely prescribed in Japan, has been shown to be effective in the treatment of influenza virus infection, but the mechanisms of its antiviral action remain unclear. We previously demonstrated that maoto binds to respiratory syncytial virus (RSV) spike proteins, thereby inhibiting their entry into host cells. In this report, a similar experiment was done to determine if maoto and its components have an anti-infective effect on the influenza virus. Our results indicate that maoto binds to the hemagglutinin (HA) spike protein, inhibiting virus entry into host cells in a manner analogous to its antiviral effect on RSV. This hemagglutinin-binding effect was observed across influenza A(H1N1), A(H3N2), and B viruses, highlighting the broad-spectrum inhibitory potential of maoto against diverse viral strains. Furthermore, maoto, internalized by cells along with the influenza virus, binds to a cap-dependent endonuclease (polymerase acidic [PA] protein) that is crucial for viral replication and inhibits its nuclease activity. Among maoto's constituent crude drugs, Ephedrae Herba (EH) and Cinnamomi Cortex (CC) were found to bind to both hemagglutinin and PA, indicating that they are responsible for the anti-infective effect of maoto. Maoto is distinctive in its multiple points of antiviral action, exhibiting a broad spectrum of antiviral properties, which makes it a versatile therapeutic agent against various viral mutations. IMPORTANCE The influenza virus is a formidable pathogen responsible for global pandemics that claim over 300,000 lives annually. Employing an ingenious evolutionary strategy, this virus undergoes constant mutation, deftly evading the action of therapeutic agents and sustaining its relentless impact. Maoto, a traditional herbal medicine, has long been known for its efficacy against viral infections and is frequently prescribed in Japan for the treatment of influenza; however, the precise mechanisms of its action remain unclear. Our study was

done to elucidate the antiviral mechanisms of maoto against the influenza virus, presenting data that supports its unique potential as a therapeutic agent capable of flexibly adapting to mutations of the influenza virus. These findings pave the way for the development of new drugs and the expansion of therapeutic options.

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Keywords: influenza virus; cap-dependent endonuclease; haemagglutinin; maoto; Ephedrae Herba; Cinnamomi Cortex

46. Julia Romanova, Artem Krokhin, Boris Ferko, Dirk Pleimes, Eva Vareckova, Frantisek Kostolansky, Andrej Egorov, **Protective efficacy of the UniFluVec influenza vaccine vector against the highly pathogenic influenza A/Indonesia/5/2005 (H5N1) strain in ferrets**, Vaccine, Volume 65, 2025, https://doi.org/10.1016/j.vaccine.2025.127795.

ABSTRACT

The emergence of new influenza strains with unpredictable antigenic properties poses a significant vaccination challenge. The increasing incidence of human H5 infections underscores the urgent need for effective pre-pandemic vaccines.

Methods

The UniFluVec and UniFluVec-wtNS1 viruses were designed as H1N1pdm vaccine candidates. Both viruses contained a heterologous A/Singapore/1/57-like (H2N2) NEP gene, which served as an attenuation factor. UniFluVec additionally carried a truncated to 124 amino acids NS1 gene, and an insertion of conserved influenza sequences. UniFluVec-wtNS1 retained the wild-type NS1 gene. The impact of NS1 and NEP modifications on attenuation and phenotypic markers was assessed in cells and mice. Safety and prophylactic efficacy were assessed in ferrets following a single intranasal immunisation with the maximum feasible dose (8.7 log10 EID50), followed by challenge with the highly pathogenic avian influenza virus (HPAIV) A/Indonesia/5/2005 (H5N1).

Results

Modifications in NS1 and NEP independently and synergistically induced a temperature-sensitive phenotype and enhanced type I/II interferon response, resulting in a highly attenuated vaccine profile. UniFluVec, incorporating both modifications within the NS

genomic segment, demonstrated superior viral clearance, reducing lung damage, and ensuring 100 % survival in infected animals.

Conclusion

The replication-deficient UniFluVec vector demonstrates safety, immunogenicity, and protective efficacy against the heterologous HPAIV strain in ferrets following a single intranasal administration.

Keywords: Live influenza vaccine; Avian influenza; Ferrets; Challenge model; Replication-deficient virus; UniFluVec vector

47. Bryan S. Kaplan, Carine K. Souza, J. Brian Kimble, Meghan Wymore Brand, Tavis K. Anderson, Phillip C. Gauger, Daniel R. Perez, Amy L. Baker, **A neuraminidase-based inactivated influenza virus vaccine significantly reduced virus replication and pathology following homologous challenge in swine**, Vaccine, Volume 46, 2025, https://doi.org/10.1016/j.vaccine.2024.126574.

ABSTRACT

Influenza A viruses (IAV) of subtypes H1N1, H1N2, and H3N2 are endemic in US domestic swine populations and contribute to significant economic losses annually and pose a persistent pandemic threat. Adjuvanted, whole-inactivated virus (WIV) vaccines are the primary countermeasure to control IAV in swine. The compositions of these vaccines are matched for hemagglutinin (HA) strain and content, often ignoring the other IAV glycoprotein, the neuraminidase (NA). The IAV NA is immunogenic and antibodies targeting epitopes adjacent to the active site have been shown to inhibit the sialidase activity of NA thereby reducing virus replication and shedding. To assess the ability of neuraminidase inhibiting (NAI) antibodies induced from WIV administration to protect swine from challenge with IAV containing homologous and heterologous NA, we produced WIV composed of viruses with an irrelevant mismatched H9 HA but expressing NA proteins from two predominant clades (N2–2002A.2 and N22002B.2) currently circulating in US domestic swine populations. Pigs that received two doses of H9N2 WIV developed vaccine-specific neuraminidase inhibition antibodies and when challenged with a wild-type H3N2 virus containing homologous NA, displayed reduced virus shedding in the upper respiratory tract and decreased virus titers in the lung compared to unvaccinated controls. Pigs challenged with H3N2 containing a heterologous NA also had reduced virus titers in the nasal swab and BALF samples. Together these results show that NAI antibodies cross-protected across phylogenetic clades and reduced virus replication and shedding in swine.

Keywords: Influenza; Swine; Neuraminidase; Vaccine

48. Alisse Hannaford, Muneerah Aleissa, Amy C. Sherman, **Update on Influenza**, The American Journal of the Medical Sciences, 2025, https://doi.org/10.1016/j.amjms.2025.10.010.

ABSTRACT

Influenza continues to challenge global health systems due to its evolving clinical and epidemiological features, with significant morbidity and mortality worldwide. Vaccine development remains a cornerstone of prevention, with attention to annual strain selection and enhanced formulations such as adjuvanted, high-dose, and recombinant vaccines. Promising innovations on the horizon include next-generation influenza vaccines, multi-pathogen vaccines, and universal influenza vaccines, with new technologies employing mRNA platforms and alternative vaccine administration strategies. The role of antivirals for prophylaxis and treatment are evaluated, including synergistic effects and resistance trends. Considerations for vulnerable populations with increased risk are highlighted. This review provides a timely synthesis of current knowledge and emerging strategies in diagnostics, therapeutics, and prevention to strengthen clinical outcomes and public health responses to influenza in the United States.

Keywords: Influenza; Infectious disease; Pharmacology

49. Changjie Lv, Wanxin Wei, Jianmei Wu, Shuang Wang, Guijie Guo, **Omaciclovir suppresses influenza A virus replication via interaction with viral PA protein**, Veterinary Microbiology, Volume 312, 2026, https://doi.org/10.1016/j.vetmic.2025.110843.

ABSTRACT

Influenza A virus (IAV) is a segmented negative-strand RNA virus that causes seasonal epidemics and occasional pandemics, posing a great threat to the public health. Current vaccines and antiviral drugs can not completely protect human and animals from IAV infection due to high frequency mutations in the viral genome and the emergence of drug-resistant strains, presenting an urgent need to explore new drugs against IAV infection. Here, we identified that omaciclovir significantly suppressed the replication of IAV. In vitro studies showed that omaciclovir inhibited replication of different IAV subtypes, including H1N1, H3N2 and H9N2. Furthermore, we demonstrated that omaciclovir strikingly attenuated replication of IAV in mice, as evidenced by a lower degree of tissue injury, slower body weight loss, and better survival, than the untreated animals following IAV infection. Mechanistically, omaciclovir interacted with viral PA protein, and interfered with the activity of IAV polymerase complexes, thereby limiting the synthesis of viral RNA (vRNA), complementary RNA (cRNA), and messenger RNA (mRNA). Together, these findings characterize the antiviral property of omaciclovir against IAV in vitro and in vivo, and provide insights into the development of potential antivirals against IAV infection.

Keywords: Influenza virus; Omaciclovir; Viral polymerase complex; PA