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SCIENTIFIC OPINION

Preparedness, prevention and control related to zoonotic avian influenza

EFSA Panel on Animal Health and Animal Welfare (AHAW)* | **ECDC*** | **Julio Alvarez**[1] | **Anette Boklund**[1] | **Sabine Dippel**[1] | **Fernanda Dórea**[1] | **Jordi Figuerola**[1] | **Mette S. Herskin**[1] | **Virginie Michel**[1] | **Miguel Ángel Miranda Chueca**[1] | **Eleonora Nannoni**[1] | **Søren Saxmose Nielsen**[1] | **Romolo Nonno**[1] | **Anja B. Riber**[1] | **Jan Arend Stegeman**[1] | **Karl Ståhl**[1] | **Hans-Hermann Thulke**[1] | **Frank Tuyttens**[1] | **Christoph Winckler**[1] | **Claire Brugerolles**[2] | **Thorsten Wolff**[2] | **Anna Parys**[2] | **Erika Lindh**[2] | **Neus Latorre-Margalef**[2] | **Marie-Anne Rameix Welti**[2] | **Ralf Dürrwald**[2] | **Ramona Trebbien**[2] | **Sylvie Van der Werf**[2] | **Magnus Gisslén**[2] | **Isabella Monne**[3] | **Alice Fusaro**[3] | **Claire Guinat**[3] | **Alessio Bortolami**[3] | **Leonidas Alexakis**[4] | **Theresa Enkirch**[4] | **Olov Svartstrom**[4] | **Katriina Willgert**[4] | **Francesca Baldinelli**[5] | **Ludovica Preite**[5] | **Malin Grant**[5] | **Alessandro Broglia**[5] | **Angeliki Melidou**[4]

[1]EFSA Panel on Animal Health and Animal Welfare members
[2]ECDC external experts
[3]EFSA external experts
[4]ECDC
[5]EFSA

Correspondence: biohaw@efsa.europa.eu and ecdc.influenza@ecdc.europa.eu

The declarations of interest of all scientific experts active in EFSA's work are available at https://open.efsa.europa.eu/experts

*Both agencies contributed equally to the Scientific Opinion.

Abstract

A risk assessment framework was developed to evaluate the zoonotic potential of avian influenza (AI), focusing on virus mutations linked to phenotypic traits related to mammalian adaptation identified in the literature. Virus sequences were screened for the presence of these mutations and their geographical, temporal and subtype-specific trends. Spillover events to mammals (including humans) and human seroprevalence studies were also reviewed. Thirty-four mutations associated with five phenotypic traits (increased receptor specificity, haemagglutinin stability, neuraminidase specificity, enhanced polymerase activity and evasion of innate immunity) were shortlisted. AI viruses (AIVs) carrying multiple adaptive mutations and traits belonged to both low and highly pathogenic subtypes, mainly to A(H9N2), A(H7N9), A(H5N6) and A(H3N8), were sporadic and primarily detected in Asia. In the EU/EEA, H5Nx viruses of clade 2.3.4.4b, which have increased opportunities for evolution due to widespread circulation in birds and occasional cases/outbreaks in mammals, have acquired the highest number of zoonotic traits. Adaptive traits, such as enhanced polymerase activity and immune evasion, were frequently acquired, while receptor-specific mutations remained rare. Globally, human cases remain rare, with the majority overall due to A(H5N1), A(H5N6), A(H7N9) and A(H9N2) that are among the subtypes that tend to have a higher number of adaptive traits. The main drivers of mammalian adaptation include virus and host characteristics, and external factors increasing AIV exposure of mammals and humans to wild and domestic birds (e.g. human activities and ecological factors). Comprehensive surveillance of AIVs targeting adaptive mutations with whole genome sequencing in animals and humans is essential for early detection of zoonotic AIVs and efficient implementation of control measures. All preparedness, preventive and control measures must be implemented under a One Health framework and tailored to the setting and the epidemiological situation;

in particular, enhanced monitoring, biosecurity, genomic surveillance and global collaboration are critical for mitigating the zoonotic risks of AIV.

KEYWORDS
avian influenza, birds, highly pathogenic avian influenza (HPAI), mammals, mutations, preparedness, public health

CONTENTS

SUMMARY

Introduction, Background and Terms of Reference (ToR)

The epidemiological landscape of avian influenza (AI) has shifted significantly since the emergence of highly pathogenic avian influenza (HPAI) A(H5Nx) Gs/GD lineage in 1996. This lineage has led to widespread transmission among and between poultry, wild birds and mammals, with sporadic human cases. Mutations enhancing mammalian adaptation, spillover events to humans and other mammals that increase the likelihood of potential reassortment events between avian and seasonal or other mammalian influenza viruses, and occasional mammal-to-mammal transmission have increased concerns about the virus potentially adapting to humans and causing a global pandemic.

Given this situation, the European Commission requested that EFSA and the European Centre for Disease Prevention and Control (ECDC) provide a joint Scientific Opinion addressing two main ToRs: (1) assess the risk posed by avian influenza viruses (AIVs) and quantify the risk of mutations increasing its zoonotic potential (ToR 1) and (2) outline comprehensive measures for preparedness, surveillance, prevention, biosecurity and control (ToR 2).

Data and Methodologies

For ToR 1, a structured framework was developed to assess the zoonotic risk posed by different AIV subtypes/clades/genotypes by assessing key mutations that may enhance adaptation to mammals, particularly humans; the assessment is supported by an epidemiological report summarising spillover events to both mammals and humans. This includes a spatio-temporal analysis of these events, identifying the main subtypes involved, exposure context for human cases and the drivers influencing spillover.

The mutation analysis was conducted in four steps: (1) identifying AIV mutations linked to phenotype traits for mammalian adaptation, (2) scoring and filtering mutations based on evidence of zoonotic potential and experts assessment, (3) screening viral sequences from the GISAID EpiFlu database (https://platform.epicov.org/) (from 2000 to 2024) for those mutations and (4) characterising the zoonotic risk of the AIVs included in the analysis based on the mutations identified and their associated traits. The distributions of identified mutations and their associated phenotypic traits for mammalian adaptation were considered in terms of prevalence by subtypes, clades and geographical origin. Possible limitations, biases and uncertainties linked to this analysis were also discussed.

The opinion also provides an up-to-date descriptive analysis of human cases to support the assessment of the likelihood and impact of zoonotic transmission of different AIV subtypes/clades in humans, using data on human cases (until October 2024) and seroprevalence (until June 2024). Trends in infections, fatality risks, immunity levels and demographic patterns are described. Data from official sources and studies were validated to highlight high-risk subtypes and exposure factors.

For ToR 2, surveillance, prevention, protection and preparedness measures aimed at reducing the risk of zoonotic AIV infection are discussed using a One Health approach. The evaluation of these measures integrates the findings from ToR 1, a literature review, expert knowledge inputs and guidance provided by the European Union (EU) Agency for Occupational Safety and Health at Work (EU-OSHA), ensuring a robust and evidence-based approach. Guidance was provided about prevention, protection and preparedness measures, which were categorised based on their target setting: general public, farm environments, occupational settings with AI exposure risks, households, communities and wildlife.

Then, surveillance aspects are addressed with a focus on zoonotic risks, emphasising the detection of mutations that may enhance AIV zoonotic potential in non-human mammals, thereby increasing the risk of transmission to and among humans. Guidance was also provided for surveillance measures aiming at detecting zoonotic strains in different non-human mammalian species, which were categorised based on the conditions under which sampling and sequencing should be triggered, the types of animals targeted and the recommended sampling indications and metadata collection.

The approach considers a global perspective beyond the EU. Moreover, existing guidance on surveillance in humans and public health laboratory practices is summarised, incorporating aspects of genomic surveillance for potential human cases.

Results, Conclusion and Recommendations

ToR 1: Risk of AIV mutations towards adaptation to mammals

Mutation analysis

The mutation analysis identified 34 key mutations in AIVs that may be associated with an increase in their zoonotic potential. The 34 shortlisted mutations identified and associated with the following phenotypic traits are: increasing mammalian specificity of virus attachment to its haemagglutinin receptor (HA:156A, HA:156V, HA:186D,221D; HA:186V; HA:221D; HA:222L; HA:224S, H5 numbering), increasing HA stability in mammal's environment (HA:222L), increased activity of the viral polymerases in mammalian hosts (PA:356R; PA:552S; PA:85I; PA:97I; PB1-F2:66S; PB2:271A; PB2:292V; PB2:526R; PB2:588I; PB2:588V; PB2:591K; PB2:591R; PB2:627K; PB2:627V; PB2:631L; PB2:701N; PB2:702R), evasion of innate immunity and counteraction of mammalian restriction factors (MP1:95K; NP:100I; NP:100V; NP:283P; NP:313V; NP:313Y; NP:52H; NP:52N) and disruption of the second sialic acid binding site (2SBS) in neuraminidase (NA:399R; NA:432E). The analysis found sporadic

instances of HPAI viruses and low pathogenic AI (LPAI) viruses with multiple mutations (≥ 8) associated with a high number (at least three) of phenotypic traits. These involved mainly subtypes A(H7N9), A(H5N6), A(H9N2), A(H3N8), but also a few viruses belonging to A(H5N1), A(H10N3) and A(H10N8). In EU/EEA, in the last 3 years, H5Nx viruses of clade 2.3.4.4b have acquired the highest number of zoonotic traits (up to four), although the vast majority of viruses showed two or three traits. The recent increase in mammalian infections associated with A(H5) clade 2.3.4.4b observed in EU/EEA and globally is considered mainly the consequence of the high virus circulation in wild birds, increased environmental contamination and greater opportunity for mammal exposure, rather than specific genetic changes. However, such infections can raise the likelihood of the emergence of adaptive mutations and the accumulation of traits that increase pandemic potential, while also enhancing the likelihood of reassortment events between avian and seasonal or other mammalian influenza viruses. Adaptive changes occur variably, with polymerase activity and immune evasion being the phenotypic traits most frequently acquired by AIVs. Adaptive mutations in the HA receptor-binding domain (RBD), identified in previous pandemic viruses, remain rare. However, they have been observed in certain viral subtypes, primarily in Asia and in Africa. Besides the selected mutations studied in this document, it is important to consider that further mutations and adaptive phenotypic traits for mammals – for which limited evidence exists or which remain poorly or not investigated – may, in future studies, be demonstrated to be relevant. Moreover, additional mechanisms, like reassortment (not covered in this Scientific Opinion), could significantly affect zoonotic potential. This highlights the critical need for comprehensive genomic surveillance of AIVs in both animals and humans, focusing on whole genome sequencing and associated metadata collection, to investigate the possible emergence of mammalian-adaptive markers. Such an approach is essential to prevent emergence and contain the spread of zoonotic AIVs. Further research priorities should include: (i) developing methods for rapid characterisation of emerging viruses (e.g. bioinformatics tools); (ii) drawing up guidelines to ensure harmonisation in experimental studies to evaluate the zoonotic potential of AIVs; (iii) maintaining and updating the inventory of mutations linked to mammalian adaptation; and (iv) harmonising and standardising viral sequences and metadata fields in GISAID and other public sequence databases.

Epidemiological and seroprevalence studies in humans

The analysis of human cases of AIV infection showed the highest occurrence of certain subtypes, with A(H7N9), A(H5N1) and A(H9N2), suggesting their zoonotic potential. In addition, subtypes like A(H3N8) and A(H10N3) have been more rarely detected. Human cases remain rare, with the subtypes that have been reported to cause most human infections so far among those that have also been identified through the mutation analysis as subtypes that tend to have a higher number of adaptive traits. A(H5N1) continues to be geographically widespread, with recent cases in the USA and Cambodia, but no cases reported in the EU/EEA. Although sustained human-to-human transmission has not been observed, seroprevalence studies for H5, H7 and H9 highlight undetected asymptomatic infections, especially in occupationally exposed groups. The severity of infection varies between subtypes, with some, like H9N2, more often associated with mild disease while others, like A(H5N1), more often leading to severe disease. However, as seroprevalence data suggest that many mild cases are probably undetected, severity and fatality rates might be overestimated.

Drivers and factors influencing the adaptation of AI virus to mammals

Genetic mutations, reassortment and selective pressures are identified as intrinsic virus mechanisms that enhance HPAI viruses' ability to infect mammals. Extrinsic drivers that heighten transmission risks and increase the likelihood of spillover and consequent virus adaptation to mammals and humans include factors and events that increase contact and exposure between wildlife, livestock and humans. These include human-related activities like farming of poultry and other AI-susceptible species at high stocking density and at low biosecurity or mixed farming, deforestation, urbanisation, global animal trade and environmental and climatic changes. As a long-term measure to reduce exposure to AI virus (AIV) and virus environmental contamination, the reduction of the density of commercial farms of highly susceptible species (both poultry and farmed mammals) primarily, but not only, in AI risk areas close to wetlands, where there may be high density of waterfowl, should be considered.

Similarly, the reorganisation (redesign or relocation) of certain animal production systems with low biosecurity in such regions, and/or livestock species often moved along the production chain, which may facilitate the AIV spread (e.g. dairy cattle in the USA) should be considered.

ToR 2: Surveillance to address zoonotic risk of avian influenza

Animal health surveillance

Surveillance should focus on mammals with known exposure to AIV-infected animals, including mammals displaying clinical signs or found dead or those with unexplained clinical signs, in areas and periods of recent AIV circulation where or when infected birds and mammals have been recently reported. Considering the broad range of hosts affected by AIVs, in particular H5Nx viruses of clade 2.3.4.4b, target species for surveillance activities include wild mammals, both free ranging (e.g. wild carnivores or marine mammals) and those at wildlife rescue centres that provide opportunities for sampling during high-risk periods, farmed fur animals, e.g. mink due to their susceptibility and the potential for amplifying the virus

spread, companion animals living on or near infected farms, domestic ruminants as they may be exposed to infected wild birds, rodents and other domestic mammals that may be exposed to infected birds, both domestic and wild, and become infected. Epidemiological factors, such as connection with outbreaks in domestic or wild animals and the identification of high-risk periods in high-risk areas, should be considered for the triggering of surveillance activities. Other triggers for surveillance should include the presence of clinical signs compatible with AIV infection in a given species or the presence of unexplained deaths or syndromes. Sampling strategies depending on the population size and diversity within the epidemiological unit should consider testing at least 10–15 sick or dead animals per outbreak (close in time and space), including samples from central nervous and respiratory tissues, and whole genome sequencing (WGS) of positive samples. For small groups of animals, like companion animals infected in the household, WGS should be attempted on all positive samples. Multispecies outbreaks should involve representative sampling. On dairy farms, bulk milk testing could also be considered, although care must be taken because milk from animals suffering from mastitis does not enter the milk tank and the dilution effect might affect sensitivity. Repeated sampling in non-culled populations is essential to monitor AIV evolution over time.

Public health surveillance

Effective public health surveillance for AI requires monitoring of exposed persons at the animal–human interface to ensure early detection of zoonotic transmission. This includes active or passive surveillance, with targeted testing of occupationally exposed persons that develop symptoms, and potentially testing asymptomatic individuals based on exposure levels. Through routine influenza surveillance, all sentinel influenza-positive samples should be typed and subtyped. In regions with ongoing AI outbreaks in animals, hospitals should enhance surveillance to detect severe human cases according to the ECDC published guidance, as well as lower the threshold for influenza testing and subtyping of positive influenza A specimens. Any suspected AI sample should be referred to national reference laboratories for confirmation and WGS of positive samples; positive samples should be sent to the World Health Organization (WHO) Collaborating Centre for further characterisation. Although sustained human-to-human transmission has not been reported, vigilance is essential to detect unusual clusters of influenza cases or limited AI transmission among humans, which could signal early stages of viral adaptation, particularly during seasonal influenza peaks when reassortment risk is higher. Awareness among health-care workers and clinicians about AI symptoms, epidemiological trends and the importance of gathering data on exposure sources is critical, as well as increasing testing for influenza taking into account the epidemiological situation. Systematic collection of metadata, including symptoms, demographics and exposure sources, should be linked with viral sequences for both public and animal health surveillance to improve the understanding of infection risks and inform prevention efforts. Timely notifications of AIV infections and outbreaks are key for pandemic preparedness. Member States should follow EU and international reporting standards, with strong coordination between veterinary and public health sectors and cross-border data sharing for an effective response.

Environmental surveillance including wastewater monitoring

Environmental surveillance, such as surface water and wastewater sampling, can complement traditional AIV monitoring by detecting the virus in natural settings and human communities. While wastewater monitoring can offer cost-effective insights into AIV circulation, it should be considered as a supplementary tool. This is because it cannot reliably identify the virus source (i.e. distinguish between animal or human origins) and often cannot determine the influenza A virus subtype. Studies of its effectiveness in providing insights on AIV circulation are still limited and more work is required to determine detection thresholds and variations based on population size.

ToR 2: Preparedness, control and prevention measures to reduce the risk of zoonotic avian influenza under the One Health approach

At the farm level and in occupational settings, biosecurity is critical to reducing the risk of AIV introduction, spillover events and zoonotic transmission. Tailored biosecurity measures, including regular staff training, audits and prompt reporting of dead wild birds near farms, are recommended alongside animal (poultry) vaccination strategies to reduce virus circulation and protect public health. These measures must be supported by increased surveillance systems and robust contingency plans. Besides biosecurity, the location and structure of animal production systems should be reconsidered as a long-term measure to mitigate the risk of AI outbreaks, including the high density of commercial poultry farms, high number and or density of highly susceptible mammal species (e.g. farmed mink) and the presence of outdoor or semi-open breeding systems, the proximity to wetlands with high densities of waterfowl. AI outbreaks in farms where mammal species are bred, similar to AI outbreaks in poultry farms, require measures including isolation or culling of infected animals, proper disposal of contaminated materials, quarantine, movement restrictions and contact tracing to limit virus spread. In outbreaks, occupational health and safety authorities should be consulted; occupational health and safety measures (organisational, technical and personal protective measures) must be revised according to the workplace risk assessment and implemented, with surveillance of exposed individuals for 10–14 days and preparedness for human case management, including antiviral use for treatment and post-exposure prophylaxis according to national guidance, isolation and contact tracing. Potential vaccination with the H5 vaccine of persons occupationally, or otherwise, routinely exposed to infected animals should be

seen at this stage as an optional complementary measure based on national recommendations; currently, there is insufficient evidence to recommend zoonotic influenza vaccination in all EU/EEA countries. Awareness campaigns targeting sectors with at-risk occupational groups should be promoted, and One Health simulation exercises should be conducted to refine response strategies.

For the general public, effective communication is essential to emphasise hygiene practices and reduce exposure risks. People should avoid both contact with potentially infected animals and the consumption of unpasteurised dairy products produced in areas where AI outbreaks in dairy cattle farms have been reported. They should promptly report sick or dead wild animals. In outbreak areas, companion animals should be protected from exposure to AIV through restricted outdoor activities; feeding them unpasteurised milk or raw poultry/game bird products from outbreak areas should be avoided. While transmission of AI via food remains unlikely, standard hygiene, pasteurisation and cooking practices are sufficient to inactivate the AIV.

In backyard poultry farms, strict hygiene practices and protective measures should be encouraged, and any signs of disease or death should be reported to the authorities.

In wildlife management, a coordinated approach among all wildlife-related stakeholders is essential for AI control, including biosecurity in wildlife handling, carcass removal from the environment and proper management of aspects that may increase the density or interface between birds and mammals (e.g. artificial water bodies, habitat fragmentation, waste disposal sites). For wild animals in captivity, preventive measures should include species separation, quarantine and biosecurity protocols; for infected animals of conservation-relevant species, isolation may be preferred over culling. Surveillance and reporting of sick or dead wildlife are critical for monitoring and containment. Preparedness should strengthen stakeholder collaboration, draft wildlife management legislation and improve data collection and predictive modelling to manage risks effectively.

1 | BACKGROUND AND TERMS OF REFERENCE AS RECEIVED BY THE REQUESTOR

This work is to respond to one of the tasks of a joint mandate received by the European Commission (EC), which requests the provision of scientific advice and guidance tools to enhance preparedness, prevention and control measures to better manage the risks pertaining to animal and public health due to the current and potentially evolving avian influenza (AI) situation.

The first task was to provide a report to set out the risk assessment of the pandemic potential of AI in the current EU and global situation, analysing the risk factors that support viral evolution and adaptation to mammals, increasing the relevance of those viruses for public health. Furthermore, potential prevention and risk mitigation measures and the actions to implement them had to be listed. This was addressed in the Scientific Report published by EFSA and ECDC in April 2024 (EFSA and ECDC, 2024).

The second task was to develop the present Scientific Opinion with the following Terms of Reference (ToRs) as received from the requestor:

- ToR 1: To assess the risk posed by A(H5N1) clade 2.3.4.4b, other HPAI viruses and other AI viruses and, if possible, quantify the risk of mutation of AI viruses to HPAI viruses and to identify the factors that influence the mutation frequency of avian influenza viruses in birds and mammals towards viruses with zoonotic potential.
- ToR 2: To provide for a comprehensive set of prevention, protection and preparedness measures following HPAI A(H5N1) and other AI virus findings in birds and mammals, in particular for:

  a. surveillance indicating which avian influenza surveillance tools are most suitable and which factors need to be considered for optimising an avian influenza surveillance programme with a view of addressing zoonotic risks; and
  b. the prevention, biosecurity and control measures to reduce the risk of zoonotic avian influenza.

1.1 | Interpretation of the Terms of Reference

Besides the possible shift from low pathogenic avian influenza virus (LPAIV) to highly pathogenic avian influenza virus (HPAIV), which is already well described in the literature and which identifies the pathogenicity in poultry, in particular in chickens, and not necessarily in humans, the focus of the current risk assessment is on avian influenza viruses (AIVs) with zoonotic potential. Influenza viruses fully adapted to a specific mammalian host, such as those adapted to pigs (i.e. swine influenza viruses), are mentioned but excluded from the analysis, due to time constraints and due to the type of risk assessment needed, which is different from the one required for avian viruses; the document only focuses on AIVs.

ToR 1 is addressed in Sections 4.1–4.5 of the present document. The aim was to provide a framework to support the assessment of the zoonotic risk posed by different AI subtypes/clades/genotypes in humans by assessing key mutations that may enhance the adaptation to mammals, particularly humans. This assessment, conducted at the global level, is based on the frequency and types of mutations (risk/impact of reassortment events are not considered in the present Scientific Opinion) in various subtypes, considering relevant epidemiological data (e.g. animal species, geography, environmental conditions). An up-to-date descriptive analysis of human cases (epidemiological and sero-epidemiological data) is presented to offer complementary evidence to inform risk assessments and surveillance, public health prevention and preparedness measures. Similarly, an epidemiological update about reported spillover cases in non-human mammals is reported, as well as a description of drivers for AIV adaptation to animal mammals and humans.

The World Health Organization's (WHO) Tool for Influenza Pandemic Risk Assessment (TIPRA) offers a structured methodology to continuously evaluate the relative risk of influenza A viruses with pandemic potential (WHO, 2020). Assessment of the zoonotic risk of circulating AIVs, i.e. the potential to spill over, cause human infections and start spreading among humans, is an ongoing and dynamic process that relies on continuous updates from various streams of data, including up-to-date epidemiological and virological information from both animal and human cases. As previously discussed in the ECDC/EFSA document 'Drivers for a pandemic due to AI and options for One Health mitigation measures' (EFSA and ECDC, 2024), assessing the potential for a zoonotic virus to cause a pandemic involves further analyses to evaluate transmissibility of the specific strain in animal models and between humans, human adaptation mutations, population immunity and cross-reactive immune responses, global capacity to timely identify non-seasonal influenza viruses in the population and to contain the initial outbreaks in humans, access to/effectiveness of vaccines and therapeutics, etc. The WHO has recently published the TIPRA assessments for AI subtypes: A(H5N6) clade 2.3.4.4, A(H5Nx) clade 2.3.4.4, A(H5N1) clade 2.3.2.1c, A(H9N2) lineages Y280 and G1, A(H7N9), A(H1N1), A(H1Nx) lineage 1C (Yamaji et al., 2024); a summary of the latest WHO TIPRA assessment is provided. ECDC/EFSA continues to assess the zoonotic risk of circulating and emerging AIV strains in the quarterly ECDC/EFSA monitoring report, and an updated risk assessment for EU/EEA is provided (EFSA, ECDC and EURL, 2024a).

ToR 2 is addressed in Section 4.6, which is about surveillance with the view of addressing zoonotic risks; the focus is on surveillance to detect mutations in non-human mammals that may increase AI zoonotic potential (i.e. leading to increased risk of transmission to and among humans). Guidance is provided for surveillance schemes for different animal categories

to detect zoonotic mutations and on which animal species to focus, considering the risk of spillover events in each species and the feasibility of the surveillance for Member States (MSs), to face spillover outbreaks, as happened in, for example, a mink farm in Finland in 2023 (Lindh et al., 2023). Moreover, already published guidance for surveillance in humans and for public health laboratories is summarised, including aspects of genomic surveillance in the event of human cases.

Prevention, protection and preparedness measures to reduce the risk of zoonotic AI (ToR 2.b, addressed in Section 4.7) are discussed under a One Health approach categorised as measures for the general public, to be applied in the farm environment and occupational settings with exposure to AI, in the household and different community settings and in wildlife. The points already addressed in the report issued by EFSA and ECDC (EFSA and ECDC, 2024) are expanded.

2 | INTRODUCTION

The epidemiological situation of AI has changed markedly over the past decades, in particular due to the emergence of the HPAI A(H5Nx) goose/Guangdong (Gs/GD) Eurasian lineage in 1996. This has resulted in a massive spread among poultry and wild bird populations, in spillover to several mammalian species, both domestic and wild and sporadic transmission to humans (EFSA, ECDC and EURL, 2024c). HPAI epidemics in a poultry flock can originate from mutation of LPAIVs of subtypes A(H5) or A(H7) to HPAIVs. Independent conversions have happened at least 51 times between 1959 and 2022, with clearly increasing incidence in the last decades, presumably related to the growing demand for poultry globally (de Bruin et al., 2022). Such epizootics may fade out by depletion of susceptible birds, either by mass mortality and/or by stamping out the birds in the infected establishments. HPAI A(H5N1) Gs/GD, however, continued to circulate and evolve while spreading to several continents. The main steps of the evolution of HPAI epidemics in animals and cases in humans in the last three decades are outlined below:

- Outbreaks in the last decades and initial strains: The first human cases of A(H5N1) Gs/GD were reported in Hong Kong in 1997, leading to severe illness and a high number of fatalities among hospitalised cases (6/18) (Chan, 2002). This outbreak marked the beginning of heightened global awareness and surveillance for AIVs.
- Spread and emergence of new clades/genotypes: Starting in 2003, A(H5N1) Gs/GD spread across Asia, affecting poultry industries and backyard flocks and causing several, mostly severe, human cases with high mortality among cases reported to the WHO. It became apparent that not only movement of poultry and poultry products but also wild birds could transmit Gs/GD HPAIVs over long distances when the first wild bird introductions were reported in wildlife parks in Hong Kong in 2002–2003 and outbreaks of HPAI occurred in Japan and the Republic of Korea in 2003–2004 (Chen et al., 2006).
- During 2005–2008, clade 2.2 of Gs/GD HPAI A(H5Nx) was spread by wild birds to Europe and Africa, followed by clade 2.3.2.1 in 2009–2010.
- In 2013, a novel AI strain (H7N9) was identified in humans in China. It was initially an LPAIV that evolved into an HPAIV for chickens with no observed change in terms of severity for humans. The outbreak caused 1568 confirmed human cases and 616 deaths between 2013 and 2017, all of which were linked to China (Millman et al., 2015). Most human cases were linked to exposure to live poultry markets, with few described events of limited non-sustained human-to-human transmission, similar to A(H5N1).
- Global expansion and ongoing surveillance: In 2014–2015, the spread of clade 2.3.4.4c A(H5Nx) viruses caused huge outbreaks in poultry and wild birds across Europe, Asia and North America (Kwon et al., 2023). While human cases were rare, outbreaks led to substantial culling of millions of birds and economic losses. New outbreaks of clade 2.3.4.4b occurred in 2016–2021, with the virus mutating and reassorting between avian species. Enhanced surveillance helped in the early detection of infected poultry flocks and the response, reducing human exposure.
- Since 2020, A(H5N8) (clade 2.3.4.4b), another HPAI strain, has caused significant outbreaks in poultry and wild birds in Europe, Asia and Africa (Brookes et al., 2022). Although primarily affecting birds, the strain caused sporadic human cases in Russia in 2021.[1]
- Recent developments and current situation. Clade 2.3.4.4b viruses of the N1 subtype became dominant from 2020 onwards and have continued to circulate to the present day (2024). They became endemic in wild birds, affected poultry on numerous occasions and spilt over to both wild and domestic mammals in several parts of the world, being introduced into the Americas and all the way to Antarctica. This caused epidemics in poultry and wild bird populations, as well as sporadic human cases. The large number of infections in different affected avian populations, which increased environmental contamination and the chance of mammal exposure, seed the increasing spillover events (interspecies transmission of the viruses) to mammals: This may facilitate adaptation of the virus to mammals, creating a new epidemiological situation with the potential of new virus reservoirs. Besides that, continued circulation in Southeast Asia of HA clade 2.3.2.1a and 2.3.2.1c is reported. Thus, the Gs/GD epidemic in animals is characterised by unprecedented geographic (worldwide, including polar regions), temporal (endemicity as opposed to seasonal circulation) spread and host range, with infection of a wide variety of bird and mammal species.

[1]https://www.who.int/emergencies/disease-outbreak-news/item/2021-DON313#:~:text=Description%20of%20the%20situation,A(H5N8)%20in%20humans.

Since November 2020, up to May 2024, 778 cases of HPAI A(H5Nx) infection (World Organisation for Animal Health (WOAH) data) were globally reported in 14 mammal families pertaining to five orders, 94% of the reported families belong to the Order of Carnivora (Figure 1), most probably infected after feeding on infected birds (ENETWILD consortium et al., 2024a). In addition to individual cases, mass mortalities were observed, such as those in sea lion colonies along the Pacific coast in South America (Ulloa et al., 2023).

Particularly worrying was the detection of HPAI A(H5N1) virus in domestic animal settings of farmed and companion mammals leading to high human exposure, such as the HPAI cases in cats in Poland, France, Korea, USA, Italy and Hungary[2] (EFSA, ECDC and EURL, 2024b), in dogs in Canada, Italy and Poland, and outbreaks in farmed fur animals in Spain (mink) and Finland (mink, foxes, raccoon dogs), and in goats, alpacas and dairy cattle in the USA and the recent die-off of large numbers of tigers in Asia (EFSA, ECDC and EURL, 2024b). Adaptive mutations have been detected in almost half of cases in mammals (not only in outbreak settings), while there is an indication of mammal-to-mammal transmission in certain outbreak settings (e.g. fur animal farms) (EFSA and ECDC, 2024). Given all these occurrences, further genetic adaptation of the virus to humans and subsequent human-to-human virus transmission could potentially lead to a future large-scale epidemic or pandemic.

This risk of a large-scale epidemic by a new/emerging influenza A virus is increased by potential reassortment between avian and seasonal human or other mammalian influenza viruses (EFSA and ECDC, 2024). Human adaptive mutations may be acquired via reassortment without the need for a gradual adaptation like via point mutations; this risk is highest in mammalian hosts that harbour mammalian-adapted influenza viruses (primarily humans and pigs, but also mustelids). Risks of reassortment should be reduced by preventing co-infections in humans and other mammals. In general, the higher the frequency of spillover events and the longer the transmission chains in mammalian populations, the higher the risk of adaptation of the virus to humans (EFSA and ECDC, 2024). Genomic surveillance and data sharing of virus sequences are of utmost importance to enhance preparedness and fill knowledge gaps. Nevertheless, the paucity of genomic data from certain epidemiological settings in some countries limits our capacity to better detect the transmission to new species and geographical spread processes.

The spillover infections in wild and domestic mammals, mostly following outbreaks in domestic and wild bird populations, and the sporadic cases in humans have triggered EU and national authorities to develop or adapt tools for epidemiological investigations and surveillance programmes, as well as to issue new emergency national legislations in the light of the upcoming seasonal flu season. In 2022, EFSA recommended passive surveillance in mammals in areas where outbreaks in poultry/wild birds would be detected (EFSA, 2023), and, since 2023, MSs have been requested by the European Commission to carry out surveillance for AI in mammals based on up-to-date assessments of the risk. HPAI detections in mammals can be considered an emerging disease according to Article 6 of EC Regulation 2016/429 (Animal Health Law, AHL)[3] and should be immediately reported to European Commission according to Article 257 (2) of AHL. Furthermore, it can trigger emergency measures for poultry and other AI-susceptible animals, such as movement restrictions, traceability and culling/disposal of animals on the affected farm. According to Article 9 of EC Regulation 2020/689, suspected cases are considered those showing clinical or post-mortem signs or laboratory tests indicative of AI, and for which an epidemiological link with a confirmed case has been established.

Considering the above, the objective of the present Scientific Opinion is to provide a framework for assessing the zoonotic risk posed by different AIVs by assessing key mutations, considering the factors that influence the frequency of these mutations and the virus adaptation to mammals and humans. A descriptive analysis of epidemiological data from human cases and human seroprevalence studies is presented to offer complementary evidence to inform risk assessments and public health prevention and preparedness measures. In this document, the prevention and mitigation measures to prevent and control a possible zoonotic AI spread are presented and discussed following a One Health approach.

3 | DATA AND METHODOLOGIES

3.1 | Assessment of zoonotic mutations of AI

The risk of mutation posed by AIVs (ToR 1) is assessed by genetic analysis of mutations and available sequences from animals and humans.

The genetic analysis was composed of the following steps: (1) identification of genetic mutations with phenotypic effect related to mammalian adaptation through an outsourced EFSA literature review, (2) screening of AIV sequences available in public databases for the identified genetic mutations, (3) assignment of a score of evidence to mutations with zoonotic potential and (4) characterisation of the zoonotic risk of AIVs through screening of mutations in virus isolates.

Preliminary work on the mutation analysis was conducted by a contractor of EFSA (Istituto Zooprofilattico Sperimentale dell'Abruzzo e Molise, IZSAM); the details are provided in a technical report (Puglia et al., 2024), which answers the following ToR:

[2] https://adozona.hu/altalanos/Madarinfluenza_fertozes_macska_Nebih_96DJFF.

[3] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32016R0429.

- To identify zoonotic markers from the scientific literature; to extract part of the information from the review by Suttie et al. (2019); and to expand the list of mutations to include as much as possible relevant markers.
- To screen all the AIV sequences available, including the ones obtained from spillover events in mammals (also humans) for zoonotic markers.

  – To generate a database in the form of spreadsheets, encompassing all the viruses presenting zoonotic markers along with the list of mutations found in each specific virus and associated metadata: virus subtype, clade (for H5 subtype), host species, location and collection date.
  – To provide a mutation frequency overview per mutation, per subtype, per species and per geographical area.

3.1.1 | First step: Identification of AIV zoonotic mutations

A comprehensive literature review was conducted in June 2024 to identify genetic mutations with a known phenotypic effect related to mammalian adaptation (Puglia et al., 2024). In brief, the literature review initially focused on the zoonotic marker inventory provided by Suttie et al. (2019). To encompass a broader list of point mutations, e.g. targeting those in the NA and NP genes, which were not covered in that review, and to cover the temporal gap between 2000 and 28 May 2024, we formulated a search string (influenza A virus [MeSH Terms]) AND (mutation OR mutagenesis OR virulence [MeSH Terms]) AND (mammalian adaptation), performed this search on PubMed for the selected period (2020–2024), and a total of 113 records were included plus other relevant papers, that were not initially selected by the search string. After the analysis of the screened records, zoonotic mutations were identified and used to expand the tables from the inventory by Suttie et al. (2019). Point mutations and molecular markers affecting phenotypic function from avian-origin influenza viruses, including those isolated from human cases, were used to generate a database for each segment as spreadsheets. For ease of understanding, HA mutations were numbered according to the H5 reference sequence A/Vietnam/1203/2004 (H5N1) (GISAID acc. n. EPI1990181) and to the H3 reference sequence A/Aichi/2/1968 (H3N2) (GISAID acc. n. EPI130007), while NA and internal proteins were numbered according to the genome segments of H5N1 A/Goose/Guangdong/1/1996 (NCBI Reference Sequence PB2: NC_007357.1, PB1: NC_007358.1, PA: NC_007359.1, NP: NC_007360.1, NA: NC_007361.1, MP: NC_007363.1, NS: NC_007364.1).

For each mutation, the following information was recorded:

- the specific mutation, as the exact amino acid substitution/deletion according to the references;
- the AIV subtype in which the mutation was originally observed;
- the phenotypic effect related to mammalian adaptation resulting from the mutation;
- the study type (either in vitro when the mutation's effect was solely described in vitro, in vivo when the mutation was supported by experimental evidence, or 'Observed' when the mutation's effects were observed through genomic characterisation);
- the corresponding scientific reference.

The phenotypic traits of the marker were described by discriminating effects on:

- evasion of innate immunity and counteraction of mammalian restriction factors,
- increased mammalian specificity of the viral polymerase (or its activity in mammalian cells),
- increased mammalian specificity of virus attachment to receptor (receptor preference),
- increased HA stability in the mammalian environment (decreased pH of fusion, increased thermal stability),
- disruption of the second sialic acid binding site (2SBS) in neuraminidase,
- increased virulence in mammals (as indicated in the literature source, it was reported if no other specific phenotypic traits were mentioned). This trait is not further considered in the analysis.

From the selected records, 592 potential zoonotic mutations were identified and listed in eight different spreadsheets (one for each viral segment PB2, PB1, PA, HA, NP, NA, MP, NS) (Puglia et al., 2024), summarising the point mutations at the amino acidic level, the subtype in which the marker was initially observed and its related phenotypic traits.

The spreadsheets summarising the identified AIV zoonotic mutations can be accessed through publicly available links contained in the technical report (Puglia et al., 2024).

3.1.2 | Second step: AIV sequences screening on public databases

In July 2024, all sequences of AI isolates collected from 1 January 2000 to 31 May 2024 were extracted from the public sequence database GISAID and screened for the genetic mutations identified in Section 3.1.1, including those from spillover events to mammals. Additional sequences from the current A(H5N1) dairy cattle outbreaks were obtained from GitHub ('avian–flu–USDA–cattle' GitHub section), to include data from AIV-infected cows missing in GISAID.

Data generated from the zoonotic markers screening in the AIV sequences were combined with metadata and elaborated to provide a mutation frequency overview per mutation, subtype, species and geographical area. The spreadsheets summarising the mutation frequency can be accessed through publicly available links contained in the technical report (Puglia et al., 2024).

3.1.3 | Mutations present in pandemic representative strains

The genetic mutations identified in Section 3.1.1 were further investigated and characterised as if observed in previous pandemic representative strains. The following viruses were selected from GISAID to represent the previous pandemic strains (Table 1).

TABLE 1 Representative virus strains used for assigning the variable 'determinant for pandemic' to mutations.

Isolate name	Isolate ID	Pandemic	Year(s)	Subtype
A/New_York/1/1918 and A/United Kingdom/1/1933[a]	EPI_ISL_1212 and EPI_ISL_69816	Spanish flu	1918	A(H1N1)
A/Singapore/1/1957	EPI_ISL_70062	Asian flu	1957–1958	A(H2N2)
A/Hong Kong/001/1968	EPI_ISL_17805317	Hong Kong flu	1968–1969	A(H3N2)
A/California/04/2009	EPI_ISL_376192	H1N1 pandemic	2009	A(H1N1)pdm09

[a]A/New York/1/1918 was used as a reference for HA and A/United Kingdom/1/1933, a suboptimal reference, was subsequently used to complement the rest of the segments. To mitigate the impact of the reference selection, the final list of mutations (13 and 40) from the mutation analysis was checked and only one was found to be impacted; 432E in NA was not present in the more appropriate reference A/BrevigMission/1/1918 virus sequence (EPI_ISL_1211) that would better represent a virus from the initial phases of the 1918 pandemic.

The segments of A/goose/Guangdong/1/1996 A(H5N1) were used as a reference. Each mutation was annotated in their respective segments and positions. For HA, H5 numbering was used, i.e. position one was regarded as the first amino acid after the signal peptide (MEKIVLLLAIVSLVKS). Alignments of each segment were produced using the CLC Genomics Workbench 24.0.2 (QIAGEN) using the very accurate alignment mode. The presence and absence of mutations were determined by inspecting the alignment of the mutation annotations. Presence in at least one of the pandemic representative strains was recorded.

3.1.4 | Third step: Assignment of score of evidence to genetic mutations

A score was assigned to each mutation to characterise the strength of available evidence (score of evidence) for its zoonotic impact. This scoring was based on (1) the type of study (in vivo, in vitro) demonstrating its biological effect, (2) whether the effect was observed in more than one virus subtype and (3) its involvement in previous pandemic events (from Section 3.1.3).

In detail, the score for each mutation was determined based on whether the mutation has been:

- Tested in in vitro studies: providing experimental data on the mutation's effect in controlled laboratory experiments (one point).
- Tested in in vivo studies: confirming the mutation's impact in studies in mammals, providing a higher level of validation (one point).
- Described in the literature in more than one subtype: providing evidence of the generic effect irrespective of virus subtype/lineage (one point).
- Detected in previous pandemics: indicating that the mutation was associated with past pandemics, adding further evidence of zoonotic risk (one point).

The final score of each mutation was computed as the sum of fulfilled criteria. The categories for evidence strength are as follows:

- Very high evidence: Mutations meeting four criteria out of four (evidence score of 4).
- High evidence: Mutations meeting three criteria out of four (evidence score of 3).
- Medium evidence: Mutations meeting two criteria out of four (evidence score of 2).
- Low evidence: Mutations meeting one criterion out of four (evidence score of 1).

This score provided a measure of the level of evidence each mutation has in terms of its contribution to mammalian adaptation or zoonotic potential.

The output from this step is a list of mutations with the respective score of evidence and the phenotypic traits. Mutations with a high and very high score were highlighted and validated by expert knowledge by a panel of nine experts

on molecular virology from the public health and animal health fields, who validated the relevance of the mutations and checked for any missing relevant mutation or inconsistencies and provided indications for considering further mutations (irrespective of their score of evidence). Experts from the public health side were selected based on a request for an expression of interest among the members of the ECDC respiratory virus surveillance network that contributed to the development of the Scientific Opinion. Experts from the animal health side were selected from EURL on AI and from expressions of interest from experts from national reference laboratories on AI in Europe.

Mutations fulfilling the following criteria were further considered in the analysis and for creating the list of relevant mutations:

- mutations with evidence score 4;
- mutations with evidence score 3, also observed in previous pandemic strains (even without indication by any expert);
- mutations with evidence score 3 and considered as relevant by at least one expert, even without being observed in previous pandemic strains;
- all the mutations considered as relevant by at least two experts, irrespective of the score assigned.

3.1.5 | Fourth step: Characterisation of molecular-based zoonotic risk in avian influenza viruses

The zoonotic risk and mammalian adaptation of AIV cannot be fully understood by examining individual mutations/genetic markers as viruses consist of multiple genes that may carry several mutations. Therefore, only sequences with all eight gene segments from AIV responsible for spillover events to mammals were analysed to capture the zoonotic potential of each virus. In this step, the distribution of mutations of interest was then described across the viruses' downloaded sequences, in those for which all the eight gene segments were available. The results are discussed in terms of frequency of mutations and phenotypic traits in subtypes and clades.

A subtype-based and not genotype-based risk assessment has been conducted, given the high frequency of reassortment events and the high genetic diversity among AIVs. Influenza A virus can in fact evolve through genetic drift and shift. Genetic shift (reassortment), which can cause major changes in the biological characteristics of a virus strain, has not been covered in this Scientific Opinion.

3.2 | Review of epidemiological data (human case detections and characteristics of human cases) and human seroprevalence data

To contribute to risk assessments evaluating the likelihood and impact of zoonotic transmission of different AI subtypes/clades in humans, and to inform relevant surveillance activities and public health interventions, this report presents an up-to-date descriptive analysis of human cases (until 31 October 2024) and seroprevalence data (until June 2024). The analysis aims to describe key features (seroprevalence, disease severity, demographic/occupational risk factors), highlight trends and assess patterns of zoonotic transmission for each AI subtype.

ECDC systematically collects information on human infections by novel influenza virus subtypes. This information is presented in the ECDC/EFSA quarterly monitoring reports,[4] as well as the weekly ECDC Communicable Disease Threat Report.[5] The epidemic intelligence process for compiling the line list of human infections from various AI subtypes involved the following procedures.

3.2.1 | Data collection via screening of publicly available sources and official public health entities

To identify potential cases of AI in humans, a broad range of open-access information sources were monitored. For this report, data from official sources only were used. Information was gathered directly from official entities, including National Public Health Institutes, Ministries of Health and supranational and regional public health organisations. Additionally, notifications submitted to the WHO via the International Health Regulations (IHR) mechanism were accessed through the WHO Event Information Site (EIS). These official notifications are provided by MSs and serve as verified sources of confirmed cases. Information received from these entities was prioritised for its reliability and was considered fully validated for line listing.

3.2.2 | Data validation and compilation

Once potential cases were identified through open-source screening, they were cross-referenced with official sources for validation. Validation criteria included comparing the collected case information (such as patient demographics, date of

[4] https://www.efsa.europa.eu/en/topics/topic/avian-influenza.
[5] https://www.ecdc.europa.eu/en/publications-and-data/monitoring/weekly-threats-reports.

disease onset, location, infection subtype and health outcomes) with official public health data from credible sources. Information confirmed by official bodies or provided through the IHR was deemed validated.

Validated cases were then entered into a line list, maintained by the ECDC. This line list organises essential epidemiological data on confirmed human cases of AI and serves as a key resource for ongoing surveillance and trend analysis, supporting timely risk assessments and informing public health responses.

This report covers available case-based epidemiological data collected between 1 November 2021 and 31 October 2024; the case-based reports in the ECDC line lists are limited to this time period. Aggregated historical data on cases and deaths for all subtypes that have caused human infections since 2021 have also been included. Published literature reviews were assessed specifically for a description of symptoms and exposure risks linked to each subtype. Additional information on AI cases in humans was obtained from different sources as described above. The WHO provides a weekly update of human cases.[6] CDC has summarised the reported global AI detections among humans and animals from 2013 to 2022, producing a comprehensive review and analysis of available surveillance data (Szablewski et al., 2023).

Seroprevalence studies and detection of subclinical infections in exposed individuals (seropositive individuals identified via serology testing and presence of antibodies against the different AIV subtypes) due to prior zoonotic infection can be used to further assess the zoonotic potential of a virus subtype and identify potential risk factors among humans. Findings from the systematic literature reviews/meta-analyses for seroprevalence for each of the subtypes A(H5), A(H7) and A(H9) (Chen et al., 2020; Qi et al., 2021; Wang et al., 2022) were described and a systematic literature review (ECDC outsourced activity) for additional studies published from 2020 to 2024 was performed. The criteria for inclusion of additional studies in the Scientific Opinion were at least 100 individuals tested for a given group, both haemagglutination inhibition (HI) and microneutralisation (MN) tests used, or HI confirmed by MN. Eligible primary studies are summarised in a table in Annex B and a summary of main findings is provided for each subtype. EU/EEA studies published between 2014 and 2020 and recent (2024) studies from the USA have also been summarised.

3.2.3 | Objectives of the analysis

To characterise AI subtypes based on their potential for zoonotic transmission, the analysis focuses on the following key aspects that are important to assess the risk of exposure, risk of transmission and risk of severe disease:

- Identify subtypes with an increased number of confirmed human cases reported.
- Identify subtypes associated with fatal outcomes among confirmed human cases.
- Evaluate immunity levels in the general population based on seroprevalence results.
- Describe seroprevalence in the different exposure settings to identify risk groups and assess the level of asymptomatic cases.
- Explore geographic and demographic trends in confirmed human cases.

The WHO's TIPRA offers a structured methodology to evaluate the pandemic risk posed by influenza viruses and their potential public health impacts (WHO, 2020; Yamaji et al., 2024). Assessment of the zoonotic risk of circulating AIVs, i.e. the potential to spillover, cause human infections and start spreading among humans, is an ongoing and dynamic process that relies on continuous updates from various streams of data, including up-to-date epidemiological and virological information from both animal and human cases. Assessing the potential for a zoonotic virus to cause a pandemic involves further evaluations of the transmissibility of the specific strain in animal models and to and between humans, human adaptation mutations, population immunity and cross-reactive immune responses, global capacity to timely identify non-seasonal influenza viruses in the human population and to contain the initial outbreaks in humans, access to and the effectiveness of vaccines and therapeutics, etc. The WHO has published the TIPRA assessments for AI subtypes: A(H5N6) clade 2.3.4.4, A(H5Nx) clade 2.3.4.4, A(H5N1) clade 2.3.2.1c, A(H9N2) lineages Y280 and G1, A(H7N9), A(H1N1), A(H1Nx) lineage 1C (Yamaji et al., 2024). A summary of the latest TIPRA assessments will be provided.

3.3 | Surveillance, preparedness, prevention and control measures

The ToR 2 is addressed by evidence collected from a narrative literature review by experts in the field with a deep understanding of the topic and from expert knowledge, taking into account the findings from ToR 1. EU-OSHA was consulted for preventive measures related to occupational health and safety.

[6] https://iris.who.int/handle/10665/375483.

4 | RESULTS

4.1 | Spillover events of HPAI to non-human mammals

As highlighted in the AI monitoring report (EFSA, ECDC and EURL, 2024b), considering the global situation, from November 2020 until September 2024, over 700 HPAI cases were reported in 73 non-human mammals species to the WOAH, affecting mainly wildlife, in particular carnivores (85% of the reported detections, data reported to the WOAH–WAHIS), but also domestic mammals, mainly dairy cattle (15%, substantially reports of cattle infections from US). Those spillover events to non-human mammals were mostly caused by A(H5Nx) viruses, subtypes A(H5N1), A(H5N8), A(H5N5).

Nevertheless, other subtypes have been reported in non-human mammals, such as A(H1) or A(H6) in guinea pig and muskrat in China (Zhao et al., 2024). Moreover, A(H3N3), A(H4N5), A(H4N6), A(H7N7) and A(H10N7) have been isolated from seals, A(H1N3) and A(H13N2) from whales and A(H10N7) from mink (Everest et al., 2021; Krog et al., 2015; Lang et al., 1981).

LPAIVs have also been linked to outbreaks in mammals, such as A(H9) that has caused infections in a wide range of mammals, including outbreaks in swine and farmed mink (Wang et al., 2016; Yong-Feng et al., 2017).

Out of the carnivores, several (51) species have been reported infected, the most represented belonging to families of Canidae (41%), felines (10%, including domestic cats), small carnivores such as Mustelidae, Procyonidae, Mephitidae (15%) and marine mammals like seals (16%). Interestingly also spill over to rodents [house mouse (*Mus musculus*), deer mouse (*Peromyscus* spp.) and prairie vole (*Microtus ochrogaster*)] were reported in relation to the outbreak investigation in cattle in the USA (Figure 1). Further details on the epidemiological description of HPAI cases in mammals are provided in the reports published by EFSA, ECDC, EURL (2024) available online.[7]

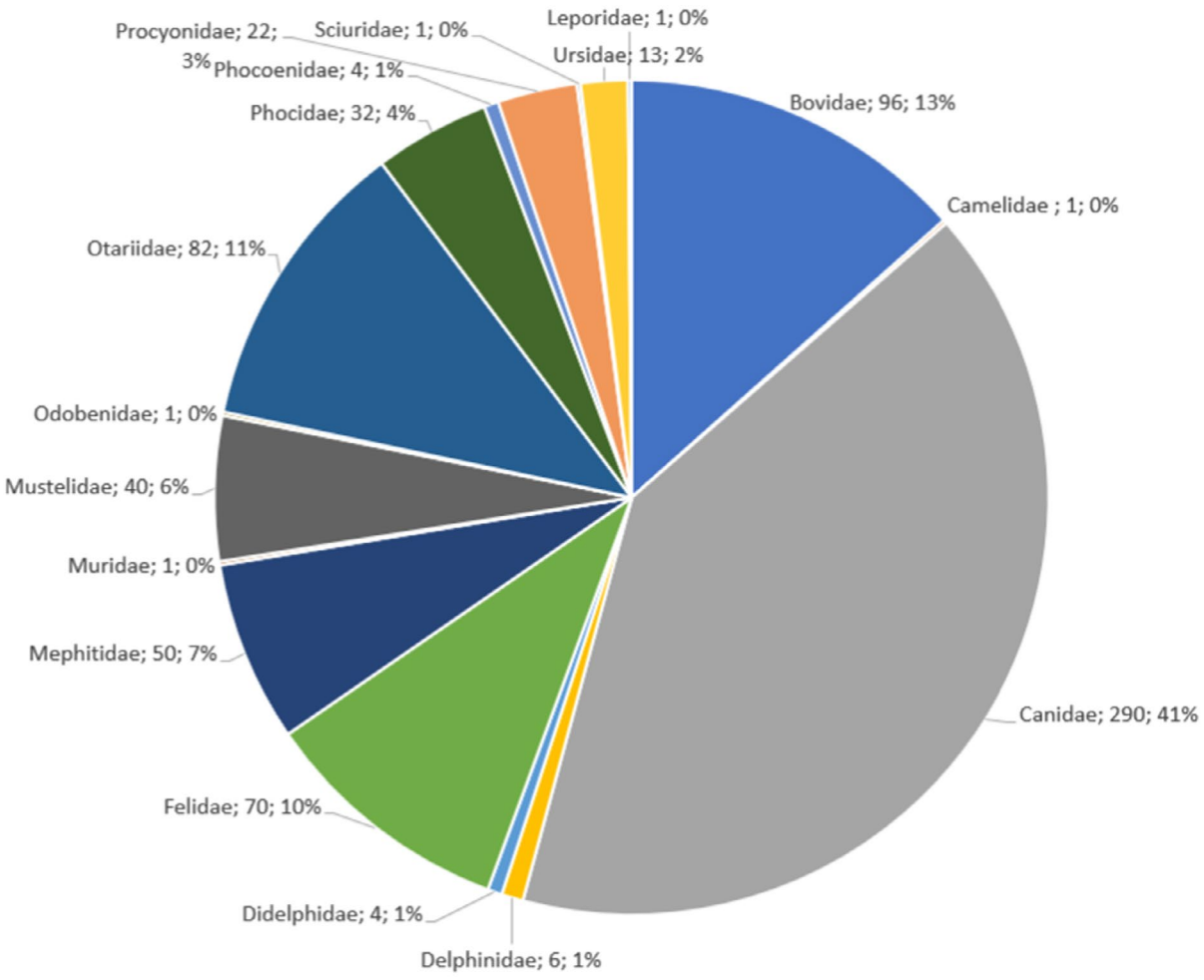

FIGURE 1 Reported cases of HPAI A(H5Nx) infection by the WOAH (90%) and national authorities (10%) in non-human mammal families and orders (November 2020 to September 2024).

One important epidemiological aspect is the continuing detection of new species infected (to date over 70 mammal species have been reported), highlighting the high virus adaptability and related large host spectrum and lack of systematic sampling (EFSA, ECDC and EURL, 2024b).

[7] https://www.efsa.europa.eu/en/topics/topic/avian-influenza.

Figure 2 reports the temporal trend at the regional level of the HPAI infection in mammals, as reported to the WOAH. In Europe, after the increase observed until the influenza season 2022–2023, the reports dropped in 2023–2024, reflecting the decline in HPAI detections in birds for the same year, both wild and domestic, thus confirming the epidemiological link between these two animal classes (EFSA, ECDC and EURL, 2024b). In the Americas, since 2022, the continuous sustained circulation of AIV in wild and domestic birds is reflected by the high number of detections in mammals during the same period.[8]

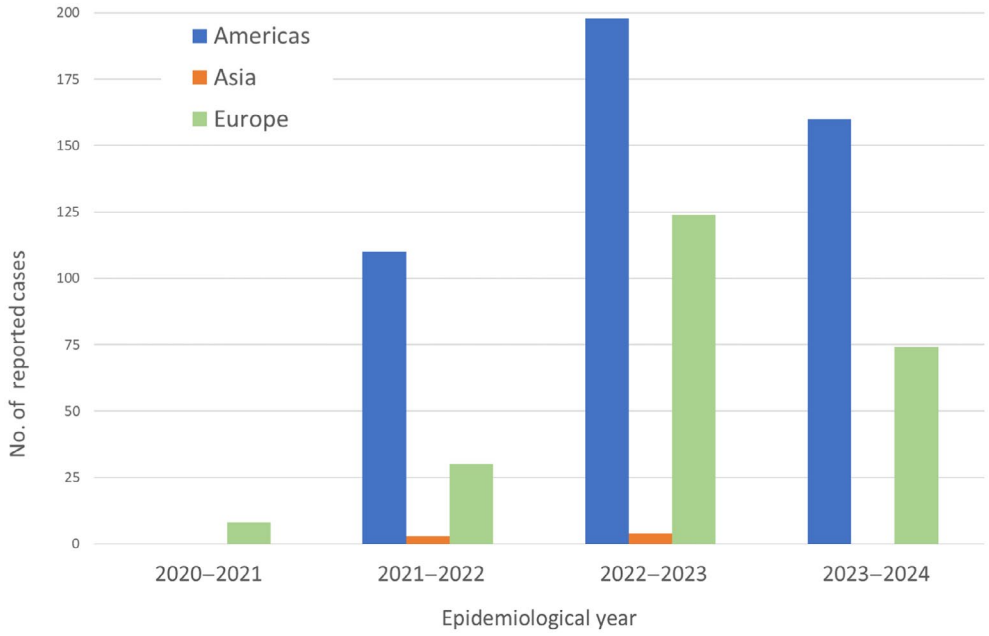

FIGURE 2 Reported detections of HPAI A(H5Nx) in non-human mammals per region and per epidemiological season (data from the WOAH–WAHIS). Note that in one single reported detection, one or more infected animal might be included.

In EU/EEA, the spillover events of HPAI (only belonging to A(H5Nx)) to mammals have been related to carnivores (i.e. cats, Eurasian otters, mustelids, raccoons, red foxes). Besides cases in wild carnivores, mostly red foxes and mustelids, the spillover events raising the most attention were the A(H5N1) cases reported in farmed mink in Spain in 2022 and in Finland in summer 2023 in American mink (*Neogale vison*), Arctic fox (*Vulpes lagopus*), raccoon dog (*Nyctereutes procyonoides*) and red fox (*Vulpes vulpes*), as well as the outbreak reported in domestic cats in Poland in 2023.

The main contribution of HPAI cases reported in mammals in 2024 are from the Americas, with a variety of wild mammals reported infected, massive outbreaks in marine mammals (sea lions) in South America and mostly the outbreaks reported in dairy cattle in the second quarter of 2024. The detection of HPAI A(H5N1) in 238 dairy cattle farms in the USA up to 25 September 2024 has driven the most attention from the media and scientific community due to concerns for possible food safety and public health repercussions. An analysis of the epidemiology and possible spread drivers, including potential routes of transmission, was presented in previously published ECDC/EFSA documents (EFSA, ECDC and EURL, 2024a, 2024b). The within-herd transmission has been characterised to be primarily through milking equipment during the milking process, and between-farm spread has probably been due to the movement of animals, farm personnel, vehicles, equipment and fomites. In response to this event, several European countries have initiated, or are planning, testing and/or surveillance of HPAIVs in cattle and milk, all of which so far have resulted in negative test results (EFSA, ECDC and EURL, 2024a).

In November 2024 in the USA, an H5N1 2.3.4.4b genotype D1.2 infection in pigs was reported for the first time, it was a backyard farm with mixed farming of poultry and livestock, including swine (two out of five were positive), where also poultry was infected.[9]

4.2 | Spillover events of AI to humans

4.2.1 | Description of AI epidemiological data from confirmed human cases

Avian influenza A viruses of different subtypes have been reported to have caused human infections historically, including subtypes A(H3N8), A(H5N1), A(H5N2), A(H5N6), A(H5N8), A(H6N1), A(H7N2), A(H7N3), A(H7N4), A(H7N7), A(H7N9), A(H9N2),

[8]https://www.aphis.usda.gov/livestock-poultry-disease/avian/avian-influenza/hpai-detections.
[9]https://www.aphis.usda.gov/news/agency-announcements/usda-animal-plant-health-inspection-service-shares-update-h5n1-detection.

A(H10N3), A(H10N5), A(H10N7) and A(H10N8). Of note, many of these viruses share a similar (H9N2-derived) internal gene cassette.

TABLE 2 Reported cases or detections of avian influenza virus in humans for subtypes of zoonotic avian influenza for which human cases have been reported since 1 November 2021.

	Cases reported since first report						Cases reported 2021-11-01–2024-10-31		
			Cases reported	Deaths		Reporting countries	Cases reported	Deaths	
Subtype	First report	Last reported	*n*	*n*	%		*n*	*n*	%
A(H3N8)	2022	2023	3	1	33	1	3	1	33
A(H5N1)	1997	2024	954	464*	50*	24	71	8	11
A(H5N2)	2024	2024	1	1	100	1	1	1	100
A(H5N6)	2014	2024	93	36	39	2	42	15	36
A(H5N8)	2021	2021	7	0	0	1	0	0	0
A(H9N2)	1998	2024	142	2	1	10	48	1	2
A(H10N3)	2021	2024	3	0	0	1	2	0	0
A(H10N5)	2024	2024	1	1	100	1	1	1	100

Note: Detections of A(H5N1) due to suspected environmental contamination (i.e. non-productive carriage/contamination) reported in 2022 (three detections) and 2023 (three detections, one inconclusive) are included. Human cases of A(H5) epidemiologically linked to A(H5N1) outbreaks at poultry and dairy cattle farms in the USA are included in the reported number of cases of A(H5N1).

*Deaths reported since 2003 out of a total of 934 cases reported.

From 1 November 2021 to 31 October 2024, there have been 168 human infections from AIVs reported by 12 countries (Australia, Cambodia, Chile, China, Ecuador, Ghana, India, Mexico, Spain (environmental contamination), UK (environmental contamination/inconclusive), USA, Vietnam). In total, seven AI subtypes have caused human infections, with A(H5N1) being the most frequently reported, followed by A(H9N2) and A(H5N6) (Table 2). Among those that caused human infections since 1997, the most frequently reported subtypes were AI A(H7N9) (1568, 55%), A(H5N1) (954, 33%) and A(H9N2) (142, 5%).

To date, human cases in EU/EEA have only been reported by the Netherlands (Fouchier et al., 2004) in 2003 and Italy (Puzelli et al., 2014) in 2013 and were of the A(H7N7) subtype; Spain has also reported two A(H5N1) cases in 2022 that were attributed to environmental contamination (Agüero et al., 2023).

The characteristics of the main subtypes causing AI infections in humans are detailed below (Figures 3–7).

4.2.1.1 | *Description of epidemiological characteristics*

Subtypes with higher occurrence of human cases in the last 3 years (2021–2024)

- A(H5N1)

There have been 954[10] human cases or detections of the AI A(H5N1) virus since the first detection in 1997. The A(H5N1) subtype has had the widest geographical spread, with human cases having been reported in 24 countries over the past decades (Figure 5). Since 2021, when the virus reached the USA and when it became endemic in wild bird populations in the EU, human cases have been reported in multiple countries, including the USA, China, Vietnam and Cambodia, with high case numbers, some of which had severe outcomes. It is important to note that the A(H5N1) virus of 1997 is genetically different from those currently circulating.

Human cases are sporadic and almost all are linked to direct or indirect exposure to infected animals or contaminated environments. From the timeline graph (Figure 4), it is evident that A(H5N1) is present with sporadic cases across the timeline, showing higher peaks in certain years correlating with known outbreaks in animals and potentially increased surveillance in response to AI activity where human spillover events are more likely. After these spikes from 2004 and up until 2015, when the highest number of human cases were reported, there appeared to be a decline. The increase in the past 2 years was mainly driven by cases in Cambodia (16 cases) and the USA (39 cases). In the USA, mammal-to-human transmission events have been identified by the A(H5N1) clade 2.3.4.4b genotype B3.13 that is causing a multi-state outbreak in dairy cattle (USDA, online-a); avian-to-human transmission has also been reported as there are also ongoing poultry outbreaks in the area. Despite the wide circulation of H5N1 clade 2.3.4.4b viruses in wild birds in EU/EEA, leading to outbreaks in domestic poultry and a high level of human exposure in occupational settings, there has been no human infection reported in the EU/EEA so far (Figure 4). Detections from Europe and the USA that were attributed to environmental contamination in individuals involved in culling activities of infected animals (and were not truly productive infections, based

[10]Human cases of A(H5) cases epidemiologically linked to A(H5N1) outbreaks in poultry and dairy cattle in the United States are included in the number of cases of A(H5N1).

on the absence of symptoms, very low viral load and the absence of specific H5 antibodies against the A/H5 virus; Aznar et al., 2023) suggest increased vigilance and surveillance in people occupationally exposed to infected animals in these regions (Figure 5).

Whole genome sequencing (WGS) data from 133 A(H5N1) human cases that have occurred worldwide were reported to GISAID, included in the genetic analysis of the Scientific Opinion (January 2000 to May 2024) and are presented in Figure 3.

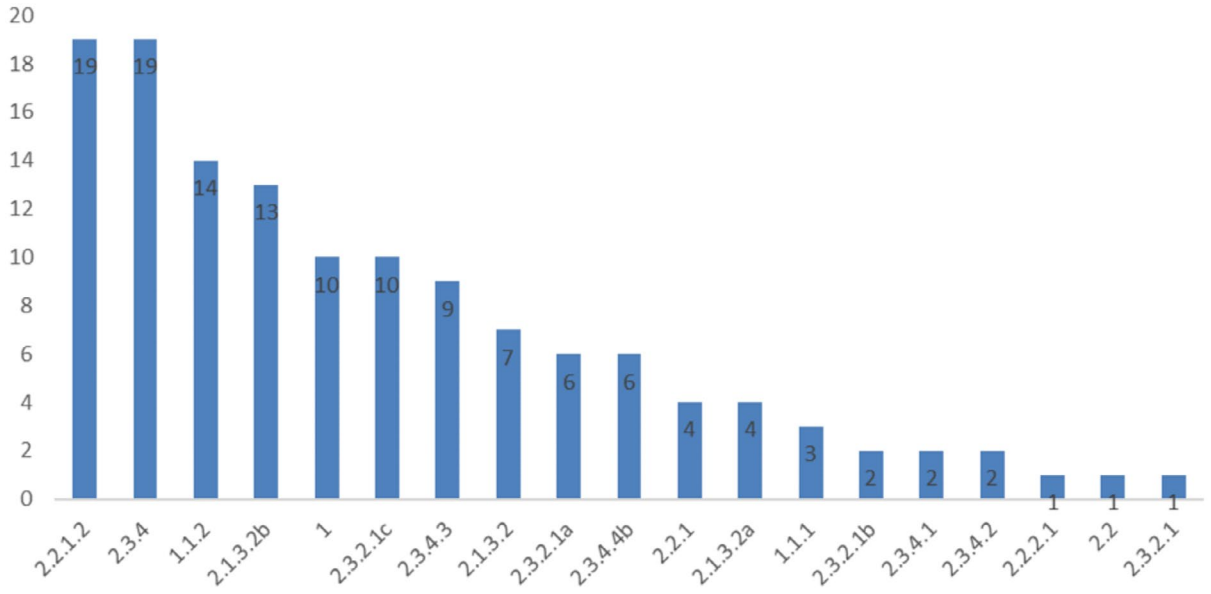

FIGURE 3 Distribution of different A(H5N1) clades among reported human cases with available sequences, January 2000 to May 2024.

Since January 2000, of the 133 submitted to GISAID sequences (15% of the total number of reported human cases), most A(H5N1) reported human cases were from clades 2.2.1.2 and 2.3.4 viruses and other clades (Figure 3); only six sequences belonged to clade 2.3.4.4b. Cambodia has faced a sudden resurgence since February 2023, caused by A(H5) clade 2.3.2.1c viruses. Fourteen cases during this period involved a novel reassortant A(H5N1) virus with gene segments from both clade 2.3.2.1c and clade 2.3.4.4b viruses (Siegers et al., 2024). Recent A(H5N1) human cases with available information (up to May 2024) all belonged to either clade 2.3.2.1c or 2.3.4.4b HA.

The infections can be mild to severe or fatal. Historically, different clades of A(H5N1) have caused numerous human cases with high mortality among reported cases (50% among reported cases from 2003 to 2024). Recent sporadic detections continue, with 71 cases reported between 1 November 2021 and 31 October 2024 (Table 2, Figure 4). Over these past 3 years, reported cases due to A(H5N1) continue to exhibit a significant mortality among reported cases (11%). It needs to be noted that only severe cases are detected in many countries in the world (and reported to the WHO) and that large numbers of mild or asymptomatic cases are probably missed. Recent human infections with A(H5N1) show a substantially lower mortality among reported cases. To date, there has been one reported fatality among human cases with known genetic clade attributed to a clade 2.3.4.4b virus. Recent United States cases associated with outbreaks in cattle and poultry farms were all mild. The cases in the USA suggest that mild and asymptomatic cases happen more frequently than severe cases but require a sensitive surveillance system and monitoring of occupationally exposed persons to potentially infected animals to identify less severe cases (Mellis et al., 2024). In addition to more sensitive surveillance that may have the capability to identify mild cases, the reduction in severity in the recently detected human cases may also be due to different exposure routes, different virus characteristics and different population groups exposed; the exposure route can play an important role in impacting the pathogenesis of the virus, as discussed later in Section 4.2.1.3.

Cases of A(H5N1) are spread across all age groups, with the 5–17 and 18–64 age groups showing a relatively higher count (Figure 7). In Egypt, the occurrence of H5 AIV was also higher in young children and adult women (Fasina et al., 2010; Van Kerkhove et al., 2011).

- A(H5N6)

There have been 93 human cases of A(H5N6) since 2014. Between 1 November 2021 and 31 October 2024, there were 42 cases reported (Table 2), many attributed to a sharp increase in cases seen around 2021, correlating potentially with larger scale outbreaks and/or more intensive surveillance efforts, and since then cases reported annually have been decreasing (Figure 5). The reduction in cases can potentially reflect the reduction in circulation of viruses in poultry resulting in less human exposures, the A(H5N6) vaccination efforts in poultry in China (vaccine was updated in 2022) and reduced surveillance for respiratory infections after the COVID-19 pandemic or also different production/consumer habits during the COVID-19 pandemic.

Apart from one case reported by Laos, all human cases have been reported by China (Figure 5).

Historically, fatality rates among reported cases have been 39%, while since 2021 mortality among reported cases was 41%.

Most cases are in the 18–64 age group, suggesting that this age group may be particularly exposed to A(H5N6), possibly due to occupational, environmental or behavioural factors (Figure 7).

- A(H9N2)

In total, 142 cases have been reported since 1998. It appears throughout the timeline (Figure 3) in smaller, more consistent numbers, potentially reflecting endemicity in poultry. A(H9N2) continues to cause human cases and has caused mild symptoms in most reported cases that have mainly been younger children. Between 2021 and 2024, there were 48 cases, although there has been a decrease in human cases reported since 2021 (Figure 4).

Human infections with A(H9N2) have been detected in 10 countries in Asia and Africa, with most cases reported from China (Figure 6).

The mortality among reported cases is low overall (two reported deaths). As the disease caused by this subtype is predominantly mild, it is likely that more mild or asymptomatic cases may have been missed. Furthermore, this virus is not notifiable in animals as it is an LPAIV that causes milder diseases in animals, individuals may be unaware of exposure to infected animals.

Interestingly, the 0–4 age group shows the highest number of A(H9N2) cases, followed by the 5–17 age group (Figure 7). This distribution could imply that children are more susceptible to (e.g. due to physiological factors, different sialic acid receptor expression, pre-existing immunity/imprinting) or exposed to A(H9N2), which might relate to environmental or behavioural factors (such as interactions with poultry in certain regions) or due to testing and reporting biases.

Other subtypes with < 10 human cases overall

- A(H5N8)

There were a total of seven cases from February 2021 reported by the Russian Federation. They were asymptomatic. There have been no reported cases since then.

- A(H5N2)

The first laboratory-confirmed human case of avian influenza A(H5N2) infection was reported in Mexico in May 2024. There has only been one human case that was also fatal. The patient, however, had multiple underlying conditions and the fatal outcome was related to the comorbidities. The patient had no known history of exposure to poultry or other animals, but the genetic analysis showed 99% similarity to LPAI A(H5N2) strains from birds in the State of Mexico.

- A(H3N8)

A(H3N8) was first detected in humans in 2022 in China, with a total number of three reported human cases, one of which was fatal. It is worth mentioning that A(H3) viruses have become established in mammalian populations on several occasions (human, equine, swine, canine).

- A(H10N3)

China has reported a total of three human cases of A(H10N3) since 2021, all of whom have recovered.

- A(H10N5)

The one and only human case was reported in 2024 in China and was fatal.

Other subtypes that have caused human infections in the past, before 2021

Few other subtypes have caused human infections prior to 2021: A(H7N2), A(H7N3), A(H7N7), A(H7N9) and A(H10N7).

The majority (55%) of all human AI infections that have been reported to date were of the A(H7N9) subtype; in total, 1568 A(H7N9) cases have been reported since 2013, with the last reports in 2019. A(H7N9) virus human infections show a prominent spike, peaking multiple times between 2013 and 2017 (Figure 4). This subtype was notably prevalent in China (all but three cases) where human infections were associated with exposure to live poultry markets. The decline/absence of cases may reflect effective interventions, such as poultry vaccination efforts. There have been no human cases reported since 2019. The severity of disease, number of hospitalisations and deaths among reported cases are high for infection with A(H7N9) (Abdelwhab & Mettenleiter, 2023; Li et al., 2019; Poovorawan et al., 2013). Early on, the virus was LPAI in poultry but still zoonotic/pathogenic in humans. The switch from LPAI to HPAI in poultry did not result in higher severity in humans.

UK (Banks et al., 1998) in 1996, the Netherlands (Fouchier et al., 2004) in 2003 and Italy (Puzelli et al., 2014) in 2013 reported 93 number of cases of the A(H7N7) subtype (Figure 4). In the Netherlands in 2003, following outbreaks in poultry, the virus was detected in 86 humans who handled affected poultry and in three of their family members. Of these 89 patients, 78 presented with conjunctivitis, five presented with both conjunctivitis and influenza-like illness, two presented with influenza-like illness and four did not fit the case definitions. Influenza-like illnesses were generally mild, but a fatal case of pneumonia in combination with acute respiratory distress syndrome also occurred. In Italy, three workers exposed to an A(H7N7) outbreak in poultry in 2013 developed conjunctivitis and tested positive for the virus.

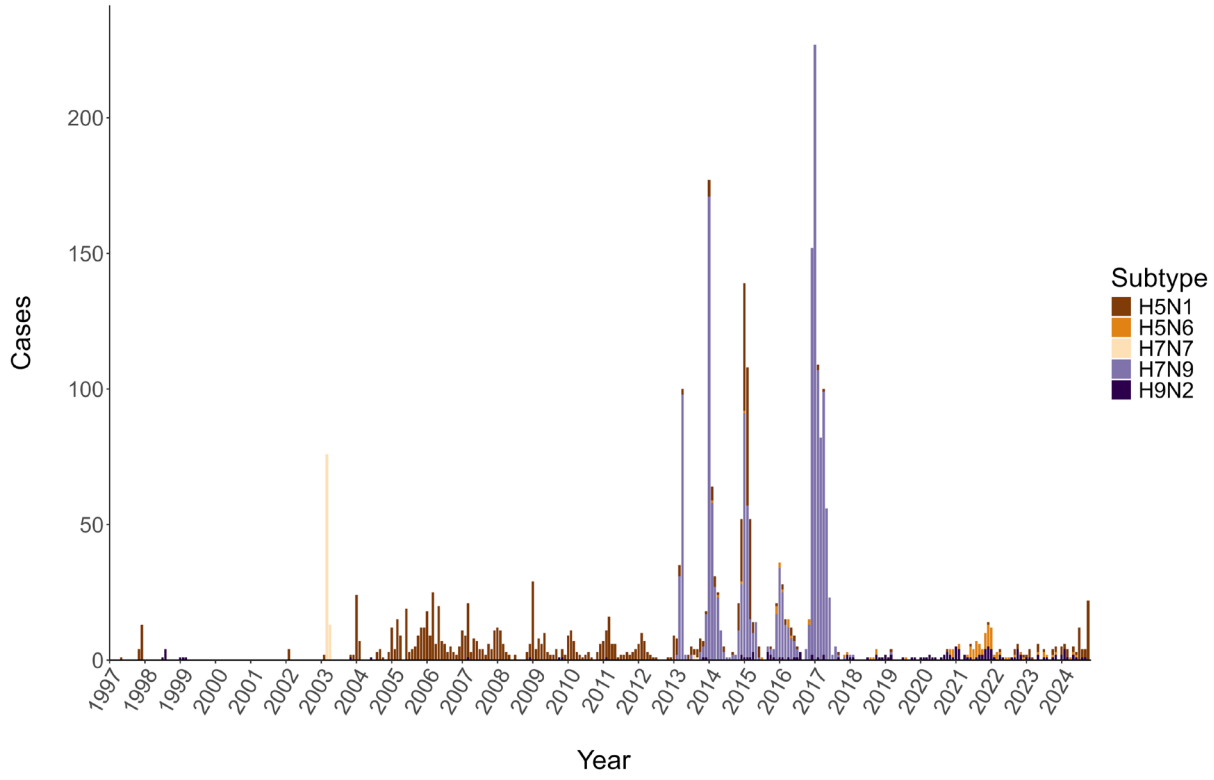

FIGURE 4 Distribution of reported cases of avian influenza virus in humans by the time of onset or detection from 1997 to 31 October 2024, including subtypes in which more than a total of 10 cases have been reported in humans. The figure includes detections of A(H5N1) due to suspected environmental contamination reported in 2022 (three detections) and 2023 (three detections, one inconclusive). Human cases of A(H5) cases epidemiologically linked to A(H5N1) outbreaks at poultry and dairy cattle farms in the USA in 2024 are included in the number of cases of A(H5N1).

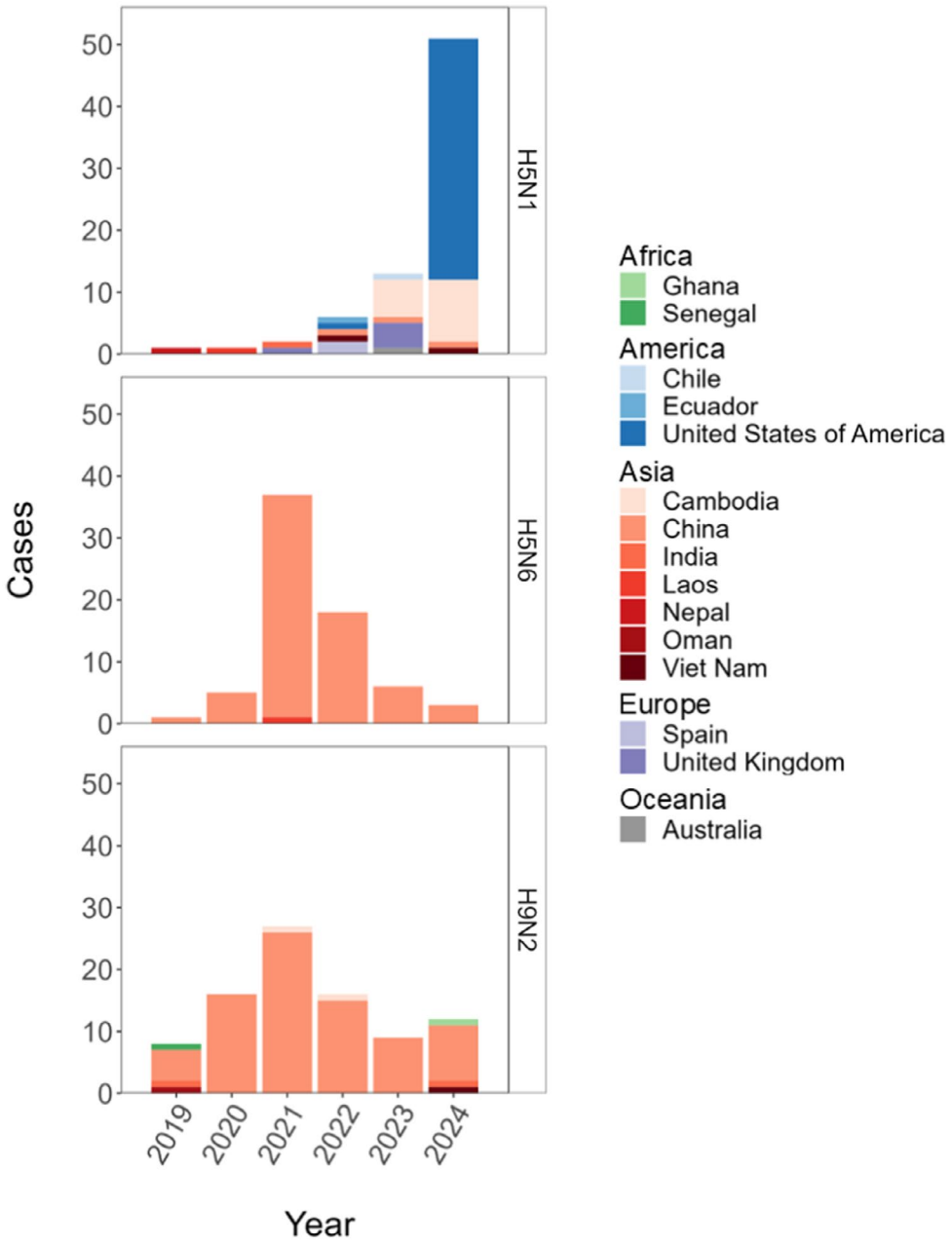

FIGURE 5 Reported cases of avian influenza virus in humans by year of onset or detection and reporting country between 2019 and 31 October 2024, including subtypes for which more than a total of 10 cases have been reported in humans. The figure includes detections of A(H5N1) due to suspected environmental contamination reported by Spain (2) and the USA (1) in 2022, and the United Kingdom (3, 1 inconclusive) in 2023. Human cases of A(H5) cases epidemiologically linked to A(H5N1) outbreaks at poultry and dairy cattle farms in the USA in 2024 are included in the number of cases of A(H5N1).

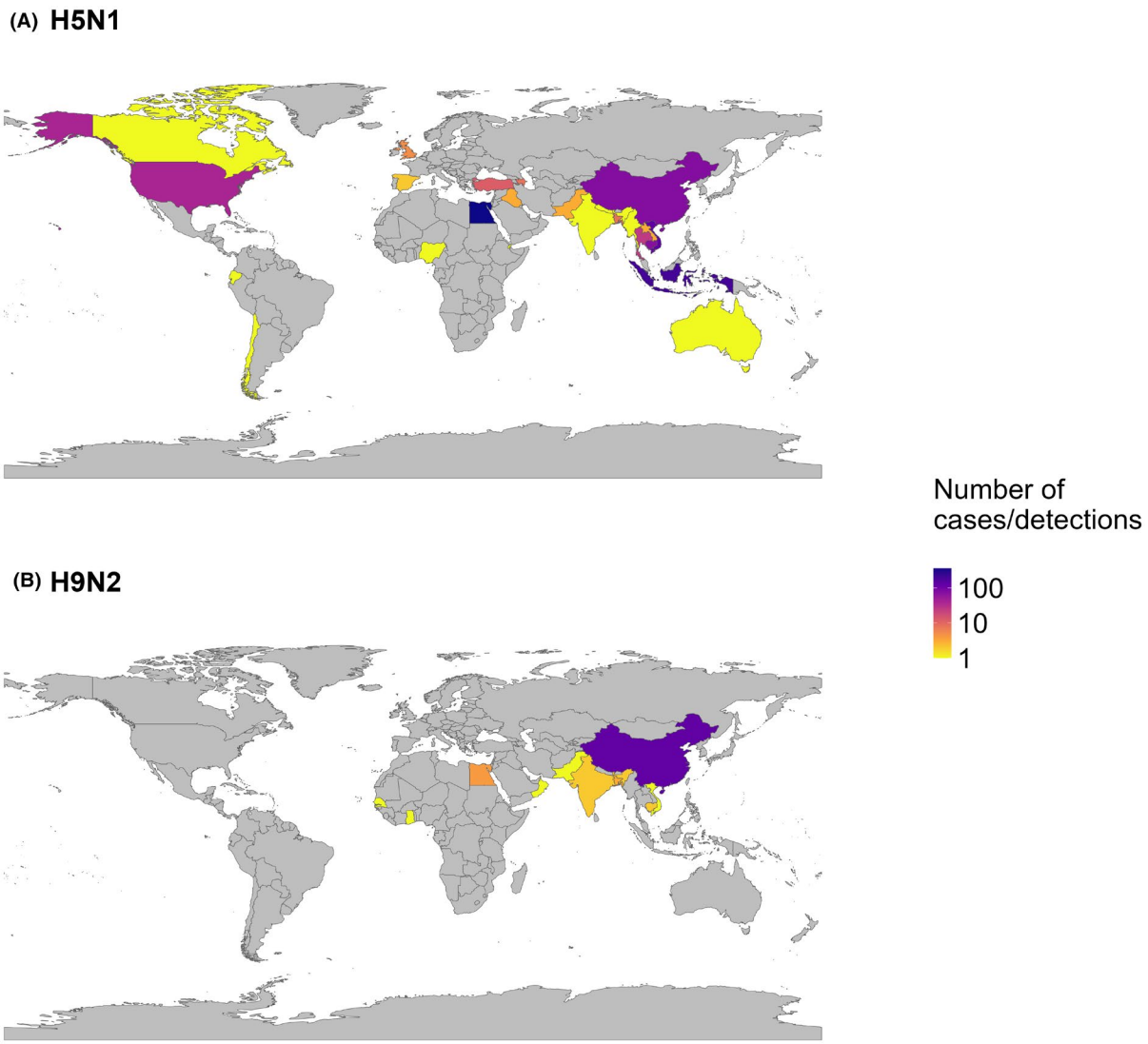

FIGURE 6 Reported number of cases or detections of avian influenza virus in humans by reporting country between 1997 and 31 October 2024 for (A) subtype A(H5N1) and (B) subtype A(H9N2). The figure includes detections of A(H5N1) due to suspected environmental contamination reported by Spain (2), United Kingdom (3, 1 inconclusive) and USA (1). Human cases of A(H5) epidemiologically linked to A(H5N1) outbreaks at poultry and dairy cattle farms in the USA in 2024 are included in the number of cases of A(H5N1).

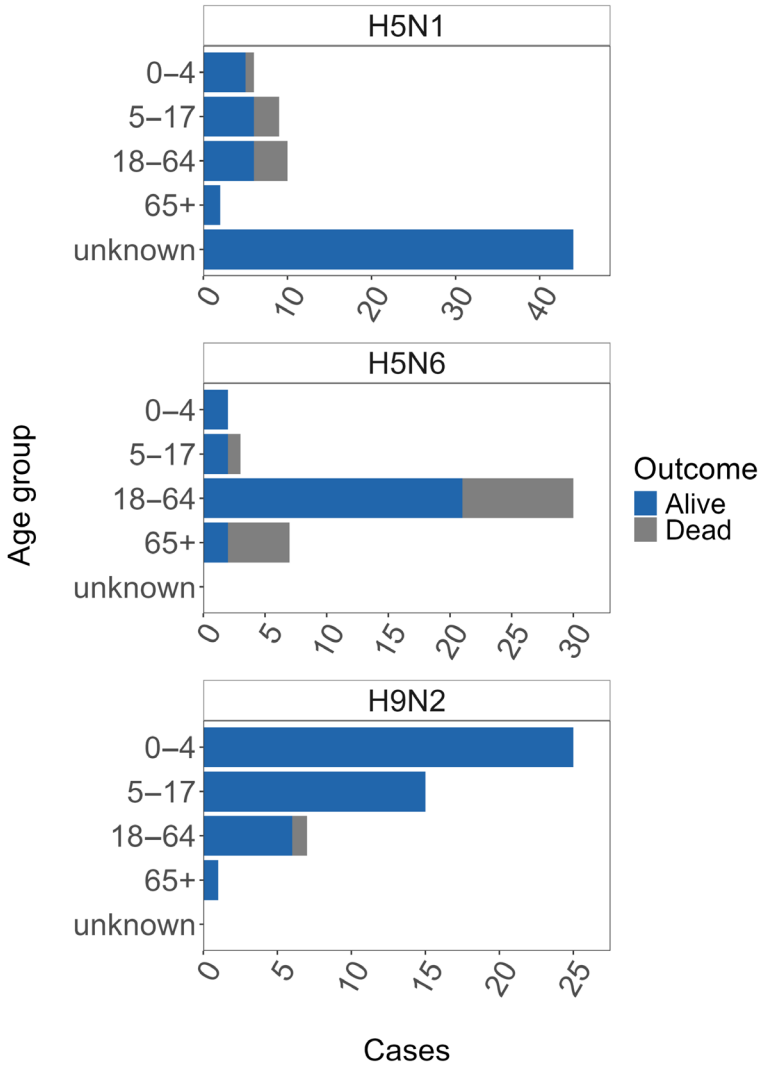

FIGURE 7 Reported cases or detections of avian influenza virus in humans by age group and reported outcome between 1 November 2021 and 31 October 2024, including subtypes in which more than 10 cases have been reported in humans. The figure includes detections of A(H5N1) due to suspected environmental contamination reported in 2022 (three detections) and 2023 (three detections, one inconclusive). Human cases of A(H5) cases epidemiologically linked to A(H5N1) outbreaks at poultry and dairy cattle farms in the USA in 2024 are included in the number of cases of A(H5N1).

4.2.1.2 | *Description of clusters of human cases*

Human-to-human transmission of AIVs has been rare so far. Limited non-sustained human-to-human transmission has been recorded in Asia for A(H5N1) before 2005 and for A(H7N9) between 2013 and 2017 in a few family clusters and health-care workers in China (Abdelwhab & Mettenleiter, 2023). Some additional suspected human-to-human transmission events were also reported in Asia after 2005 (CDC, online-c). In Europe, in 2003, human-to-human transmission of A(H7N7) was reported in the Netherlands from infected poultry workers to three of their household contacts (Bridges et al., 2000; Ma et al., 2015; Wang et al., 2019).

4.2.1.3 | *Description of exposure characteristics of human cases*

Confirmed human cases of AI infections are primarily linked to direct, unprotected contact with infected animals, especially poultry, or through exposure to their secretions and contaminated environments. Transmission to humans often occurs through droplets entering the nose or through contact with the conjunctiva, with aerosol transmission emerging as a significant risk factor during high-exposure activities. Ocular exposure is a possible route of transmission, as the eye can serve as an entry portal for AIVs, because it contains α2,3-linked sialic acid (avian-type receptor) (Belser et al., 2018; Fouchier et al., 2004).

In addition to the geographical distribution of AIVs in animals, a range of cultural, recreational and occupational activities contribute to infection risks in humans. Individuals who may be exposed directly or indirectly to infected animals, such as those working in live bird markets, farms or backyard holdings with limited biosecurity measures, slaughterhouses or those involved in outbreak response, are particularly vulnerable to infection (Bos et al., 2010; Offeddu et al., 2016). Health-care workers are also considered an occupation with high-risk exposure (Bridges et al., 2000; te Beest et al., 2010). The risks

associated with these activities, however, vary by region, leading to different levels of human exposure. For example, live bird markets are more prevalent in some countries, e.g. in Asia and Africa, and are associated with increased infection risks due to high poultry turnover, continuous contact between workers and handling of poultry with limited biosecurity measures. In backyard settings, practices also vary; in some regions, individuals may slaughter poultry for consumption showing signs of illness, and further increasing exposure risk. By contrast, commercial farms, particularly in developed countries, typically operate with higher biosecurity standards and monitoring systems, which can reduce infection exposure in workers and enable earlier detection of cases when infections occur.

Recent cases of A(H5N1) in the USA, associated with outbreaks in poultry and cattle farms, as well as seroprevalence studies, demonstrate infection risks in scenarios involving direct exposure to infected animals or contaminated farm environments. The risk of infection correlates with the viral load and the nature of exposure. Birds, as natural hosts of AIVs, shed viruses in high loads and high viral load has also been reported in milk from infected cattle (Caserta et al., 2024; Le Sage, Campbell, et al., 2024). In professional settings, exposure routes may differ from those seen in backyard poultry farming or in live animal markets, potentially affecting disease severity.

Despite substantial exposure opportunities within the EU/EEA, particularly due to recent A(H5) outbreaks in poultry, no confirmed human infections have been reported. The absence of confirmed AI cases among farm workers in EU/EEA, despite extensive outbreaks in poultry, highlights several possible explanations. Severe human cases in Asia have typically been linked to specific high-risk exposure settings, such as backyard farms or live animal markets, where biosecurity measures were minimal. In EU/EEA, by contrast, occupational exposure among professional poultry workers may be associated with better established biosecurity protocols and the routine use of personal protective equipment (PPE). Additionally, the demographic profile of professional poultry workers – mainly adults in good health – may make them less susceptible to severe disease outcomes. Mild or asymptomatic cases may be missed, as testing for AI is usually targeted towards symptomatic and high-severity cases in most countries. This limitation could mean that some mild or asymptomatic infections may go undetected in occupationally exposed groups, a factor that may also apply to other AI genotypes but only becomes evident when sensitive surveillance systems are in place that can detect milder cases (i.e. when there is enhanced surveillance and targeted testing in the animal–human interface).

4.2.1.4 | *Description of symptom range and factors potentially affecting disease outcome of AI in humans*

AI infections in humans demonstrate a range of clinical presentations, varying from asymptomatic or mild cases to severe or fatal outcomes. Asymptomatic cases may sometimes represent non-productive infections, where the virus merely contaminates the conjunctiva or respiratory tract through environmental exposure. Aerosolised viral particles can facilitate deeper respiratory tract infections, which may contribute to more severe disease.

Mild cases of AI in humans often involve self-limiting symptoms that resemble typical influenza, such as fever, coughing, sneezing or conjunctivitis. In a recent cluster among United States poultry farm workers exposed to A(H5) (2.3.4.4b) during the depopulation of infected birds, all workers who tested positive reported only conjunctivitis and mild symptoms (Drehoff et al., 2024). Similar mild manifestations have been observed in individuals exposed to infected cattle through possible mammal-to-human transmission. These cases involved high viral loads in secretions such as milk and contamination in the surrounding environment. In the recent United States experience in poultry and cattle farm A(H5N1) outbreaks, severe illness has not been observed in people exposed occupationally. Human cases, including severe or fatal cases, of AI in humans have typically been associated with close contact with infected birds or possibly highly contaminated environments.

There appears to be no direct correlation between the virulence of AI in birds and its impact on humans. For instance, LPAI A(H7N9) caused no or mild symptoms in chickens but frequently led to severe respiratory disease in human cases of A(H7N9) infection (Abdelwhab & Mettenleiter, 2023; Liu et al., 2021). Human infection associated with other influenza viruses within the A(H7) subtypes, like A(H7N7), has typically consisted of eye infection and presented with conjunctivitis (Belser et al., 2009; Puzelli et al., 2014). Conversely, the LPAI A(H9N2) virus often results in mild clinical symptoms in humans, although isolated severe cases have also occurred.

In documented human cases, virus replication is usually confined to the respiratory tract, although instances of extrapulmonary replication, including in the brain, have been reported (Abdelwhab & Mettenleiter, 2023; Korteweg & Gu, 2008; Uiprasertkul et al., 2005). The A(H5N1) clade 2.3.4.4b virus has caused neurological signs and brain infections in mammals (Murawski et al., 2024), raising questions about potential neurological impacts in human cases as well. Seasonal influenza has similarly been associated with encephalitis in some human cases.

The variability in clinical outcomes is probably influenced by a combination of factors, including the viral genotype, viral load in the exposure material, duration and nature of exposure, adequacy of PPE at the time of exposure, route of transmission, underlying health status, previous immunity and access to medical care. These items collectively shape the spectrum of disease severity observed in human cases of AI. In addition, surveillance systems and reporting biases need to be taken into account when interpreting the severity data.

Cross-reactive immunity with human seasonal viruses can be an indicator that zoonotic infections with the cross-reactive animal virus may result in milder infections, even if a pandemic may still be caused by them (such as with A(H1N1)pdm09). Interestingly, recent studies have indicated that antibodies against seasonal A(H1N1) influenza viruses might provide some level of cross-reactivity to A(H5N1) clade 2.3.4.4b through neuraminidase (NA) inhibition (Daulagala et al., 2024; Le Sage, Werner, et al., 2024), but systematic data are not yet available. The degree to which serological cross-reactivity in individuals

born before 1968 confers protection against A(H9N2) infection also remains unknown (Yamaji et al., 2024). Further studies are needed to confirm whether cross-reactive NA antibodies confer protection against AIV infection or modulate disease severity. If so, some people may have partial immunity, e.g. adults could be partially protected against A(H5N1) due to cross-reactive immunity because of previous exposure to A(H1N1) viruses (Peacock et al., 2024). In a recent study published in a preprint, it was demonstrated that mice infected with the 2009 pandemic H1N1 virus strain A/California/04/2009 or vaccinated with a live-attenuated influenza vaccine were moderately to highly protected against a lethal A/bovine/Ohio/B24OSU-439/2024 A(H5N1) virus challenge (Brigleb et al., 2024). The role of T-cell-mediated immunity needs to also be investigated; $CD4^+$ and $CD8^+$ T cells activated after contact with seasonal influenza viruses can also recognise peptides from HPAI A(H5N1) influenza virus (Lee et al., 2008; Sidney et al., 2024). In the aforementioned preprint, T-cell epitope mapping analysis revealed a high conservation of amino acid sequences within the internal proteins of the bovine HPAI A(H5N1) virus strain (Brigleb et al., 2024). It is therefore necessary to explore additional factors that contribute to protection against HPAI A(H5N1) viruses, such as memory T-cell responses, in addition to HA inhibition or neutralising antibodies.

The possible effects of original antigenic sin, or immunological imprinting – the phenomenon in which the immune system, when encountering a new but similar virus, relies on memory responses generated from the first exposure – also needs to be investigated. For influenza, when individuals are exposed to a new subtype, their immune systems might preferentially produce antibodies against epitopes of a previous flu strain they encountered in childhood, rather than responding specifically to the new virus's unique epitopes (Tesini et al., 2019). This could be both beneficial and limiting as, on the one hand, it might provide some level of protection, potentially reducing the severity of infection, as described in previous studies and in a recent preprint for older individuals that have higher antibody titres to historical and recent H5N1 strains than younger individuals (Garretson et al., 2024; Gostic et al., 2016) and, on the other hand, the immune system's focus on these familiar epitopes could prevent it from effectively responding to unique features of the novel virus, potentially limiting the efficacy of the immune response. Further studies are needed to clarify the role of this phenomenon in AIV infections.

4.2.2 | Description of human seroprevalence data for H5, H7 and H9 subtypes

There are limited data on population immunity at the general population level and in different exposure settings. Subclinical AIV infections have been studied mainly in Asia. Subclinical infections and seroprevalence estimates identified for each subtype in each study and by exposure setting are presented in Table B.1 in Annex B.

In EU/EEA, serological studies have been performed for A(H9N2) in Romania, A(H7N3) and A(H7N1) and A(H9N8) in Italy and A(H7N7) in the Netherlands (Abdelwhab & Mettenleiter, 2023), but only two studies were published after 2014 (Abdelwhab & Mettenleiter, 2023; Coman et al., 2014).

Overall, available data suggest that subclinical human AIV infections are uncommon in the general population but less so in exposed populations. In the period and areas of the studies, seroprevalence of AIV antibodies was lower in the general population for all of the three HA subtypes A(H5), A(H7) and A(H9) compared with the levels identified in exposed groups. Although the results of the studies are not generalisable, it is expected that the level of immunity in the general population will probably be very low for all of the zoonotic subtypes. Seroprevalence estimates were higher in exposed groups, indicating potential low-level unrecognised asymptomatic infections in exposed individuals. Most subclinical infections were detected in exposed individuals in live bird markets, backyard farms or commercial farms (e.g. farmers, veterinarians) that are exposed groups at higher risk of infection. Health-care workers (HCWs) have also been considered a risk group in some of the studies. The seroprevalence levels observed in exposed groups for A(H5) provide valuable context for interpreting case fatality globally, as they suggest that reported human cases, which are predominantly severe, may lead to an overestimation of case fatality for A(H5) and A(H7) subtypes. Seroprevalence estimates support the assumption that a greater number of mild or asymptomatic A(H5), A(H7) and A(H9) cases occurred than were reported. The main study findings are summarised below.

- A(H5)

A systematic literature review and meta-analysis of published serosurveys on occupationally or behaviourally exposed groups and the general population conducted in 2020 (1997–2020) showed that seroprevalence of A(H5N1) virus antibodies remained at low levels (Chen et al., 2020). Variations in seroprevalence of A(H5N1) virus-specific antibodies seemed consistent with the extent of reported exposure to A(H5N1) virus and highest among occupationally and otherwise exposed populations. Across studies, the point estimates of the seroprevalence of A(H5N1) virus-specific antibodies were higher in poultry-exposed populations (0%–0.6%) and persons exposed to both human A(H5N1) cases and infected birds (0.4%–1.8%) than the general population (none to very low frequencies). Among occupationally exposed populations, persons who worked in live poultry markets had a higher prevalence of A(H5N1) virus-specific antibodies than poultry farmers and veterinarians, probably because they were typically involved in more than one high-risk operation (e.g. butchering and processing poultry) (FAO, WHO and WOAH, 2024a). None or very low prevalence of virus-specific antibodies was detected among close contacts of confirmed A(H5N1) cases (Chen et al., 2020).

Recently published studies (2020–2024) are included in Table B.1, Annex B; their findings are consistent with those from the review (Chaudhry et al., 2020; De Marco et al., 2021; Wang et al., 2024).

Preliminary results of serology studies conducted in human samples from all 10 United States Departments of Health and Human Services from flu seasons 2021–2022 and 2022–2023 (CDC, online-a) did not identify antibodies against the current A(H5N1) clade 2.3.4.4b viruses. Shittu et al. (2024), in a preprint manuscript, presented the results of a study carried out in two dairy farms in Texas that were affected by HPAI A(H5N1), where two out of 14 dairy workers had antibodies against A(H5N1). In July 2024, in Michigan, one study of 35 dairy farm workers showed no evidence of infection (CDC, online-a). In addition, recent evidence from the USA from surveys and serologic testing to identify HPAI A(H5) infections among dairy workers in two states, indicated that 7% of participating dairy workers (8/115) had evidence of recent infection with HPAI A(H5) virus (Mellis et al., 2024). They all reported milking cows or cleaning the milking parlour and none of the workers with serologic evidence of infection used respiratory protection; three used recommended eye protection (Mellis et al., 2024). This finding provides evidence of increased seroprevalence in exposed occupational groups, similar to results from Egypt when outbreaks in live bird markets were investigated and a 4.6% seroprevalence among workers was reported (Gomaa et al., 2023).

- A(H7)

A systematic literature review and meta-analysis from Wang et al. (2022) of published serosurveys worldwide that included studies between 2013 and 2020 showed that human infections with avian influenza A(H7N9) virus have been uncommon. The median seroprevalence for the general population was 0.02% (Wang et al., 2022). Workers with occupational exposures to poultry and close contacts of A(H7N9) human cases had low risks of infection. For poultry workers, the prevalence of H7N9-specific antibodies was 0.1%, 0.4% and 0.5% when using the WHO-recommended, modified WHO-recommended and non-standardised seropositive definitions, respectively.

Recently published studies (2020–2024) are included in Table B.2, Annex B; their findings are consistent with those from the review (Chaudhry et al., 2020; De Marco et al., 2021; Horm et al., 2016). In addition, Meijer et al. showed antibodies against the avian A(H7N7) virus in an unexpectedly high proportion of exposed persons during an epidemic of A(H7N7) in animals in 2003 in the Netherlands; 49% seropositivity in 508 persons exposed to poultry and in 64% of 63 persons exposed to A(H7N7)-infected persons (Meijer et al., 2006).

- A(H9)

The seroprevalence of A(H9N2) infection among humans in different regions, demographic characteristics and exposure populations was variable. A(H9N2) serological evidence in humans has been reported from Asia (China, Cambodia, Thailand, India, Mongolia, Pakistan, Iran, Lebanon), Africa (Egypt, Nigeria), Europe (Romania) and North America (USA) (Khan et al., 2015; Qi et al., 2021). In a recent meta-analysis of 45 studies conducted in China from 1997 to 2020, including a total of 59,590 individuals, the overall A(H9N2) seroprevalence was estimated to be 5.56% in the poultry market worker group, for all occupationally exposed populations 3.18% and in the general population 1.38% (Qi et al., 2021). Another meta-analysis that covered the period 1997–2013 described similar findings for the occupationally exposed persons (Khan et al., 2015).

One additional primary study was published after the review date; the study was conducted in Pakistan in 2015–2016, during which seroprevalence in poultry was found to be 89%. The study reports higher levels of seroprevalence among occupationally exposed butchers in live bird markets of 15.5% and 6% in the general population (Chaudhry et al., 2020) (Table B.3, Annex B).

There are two studies from EU/EEA during this period; while Romania had recently experienced multiple incursions of HPAI among domestic poultry, this cohort of Romanian agriculture workers had sparse evidence of avian influenza H9 virus infections (1/23 with symptoms) (Coman et al., 2014). In the Italian study, antibodies specific to H9 (among other subtypes) were found in three out of 57 poultry workers (De Marco et al., 2021).

4.3 | Drivers and factors influencing the adaptation of AI virus to humans and other mammals

Besides the epidemiological description of cases, an essential aspect is to investigate the drivers that lead to the spillover events of HPAI from birds to mammals, and subsequently potentially also to humans, since this can steer the planning of prevention and preparedness against HPAI.

As discussed in EFSA and ECDC (2024), spillover transmission occurs when a pathogen in a reservoir population comes into contact with a novel host population or species and is successfully transmitted resulting in virus replication within the new host. It may or may not spread further within the new host population. For the HPAI A(H5N1) virus, these spillover events are often observed and this increased contact of the virus with new species increases the probability of adaptation (virus mutation), and as a consequence the probability of larger outbreaks or epidemics. Most important from the zoonotic point of view are those spillover events involving mammal species that are zoologically or ecologically 'close' to humans, thus increasing the likelihood towards human adaptation to the virus.

The risk of spillover events is influenced by several factors, which can heighten or mitigate the potential for cross-species transmission (EFSA and ECDC, 2024). These factors are mainly all those events or conditions that may increase exposure of mammals or humans to AIV leading to transmission and further spread and thus increased chance of adaptation of avian

virus to mammals. In the last decades, changes in the environment, climate and host interactions, mostly due to human activities such as agriculture, trade and shipping, travelling, urbanisation, changes in food habits, landscape exploitation, etc., are considered to drive and increase the risk of spillover and/or interspecies transmission of pathogens (Wolfe et al., 2007).

The main drivers for viral evolution and adaptation of HPAIVs to mammals and humans, either intrinsic, i.e. linked to virus and receptors characteristic of the host or extrinsic, i.e. those external factors determined by ecology or human activities, are described in EFSA and ECDC (2024) and summarised below:

Virus strain characteristics:

- Genetic mutations: The selection pressure is high in certain subtypes of AI, like certain clades of A(H5N1) and A(H7N9), which have been infecting different host species and thus adapting more easily, and increasing their potential to infect mammals, including humans. Higher selection pressure is due to factors like immune evasion, cross-species transmission, high mutation rates and the impact of human interventions (e.g. vaccination and biosecurity). These pressures drive the virus to evolve in ways that increase its fitness, making it more adaptable to new hosts, evading immune responses or developing resistance to control measures. Genetic changes can enhance their ability to bind to and enter human respiratory cells and to enhance the completion of the viral life cycle in these hosts for instance by bolstering viral replication/transcription or strongly suppressing their innate defences.
- Reassortment: The mixing of genetic material from different influenza strains can create new strains with possible human-to-human transmission and pandemic potential. This is particularly relevant when the new reassorted genes originate from a mammalian influenza virus (e.g. when an AI virus reassorts with a human seasonal influenza virus) and can potentially lead to the emergence of new pandemic strains (as observed in previous pandemics).

Human activities and human–animal interactions:

- Farming of poultry and other AI-susceptible species, especially at low biosecurity or mixed farming (coexistence of several species in the same farm), increases the likelihood of exposure of humans and other animals to infected birds and also increases the opportunities for reassortment events. Practices like live bird markets and backyard farming also pose risks due to the close proximity of different animal species.
- Farming highly susceptible species (e.g. fur animals), or at high stocking density and with low biosecurity, especially those near wetlands where waterfowl density is high, represents a risk for increasing chance of spillover and consequent virus adaptation to mammals and humans.
- All human activities that increase the contact rate between wildlife, livestock and humans, such as deforestation and urbanisation, degrade and encroach on wildlife habitat. Also, global animal trade and travel may facilitate the spread of AIVs, with infected avian and mammalian hosts potentially introducing novel strains to different regions.

Wildlife ecology:

- Wild birds can spread AI over long distances, bringing the virus into contact with domestic poultry, mammals and humans.
- Most reported cases of wild mammal infection are among carnivores, suggesting a connection between predation and scavenging behaviour and AI infection (from wild birds). Moreover, synanthropic and peri-urban species, might act as bridge hosts between wild birds, domestic animals and humans, facilitating viral evolution. Useful insight into the factors leading to the spillover of HPAI from birds to wild mammals is provided by the ENETwild consortium (ENETWILD consortium et al., 2024a). In a recent work by ENETWILD,[11] these drivers were further categorised and discussed according to the compartment they refer to:

  Virus:

  – Circulation of HPAIV subtypes/strains in birds that are more likely to infect wild mammals.

  Birds:

  – Seasonal waterbird migration;
  – Waterbird aggregation;
  – High numbers of HPAIV-infected wild birds, creating disease hotspots.

  Mammals:

  – Shared habitat with waterbirds;

[11] https://zenodo.org/records/13885776.

- Life in an aquatic environment: Mammals with aquatic lifestyles, can be exposed to the virus via contaminated waters, e.g. H5N1 spillover in harbour porpoise not expected to be associated with bird predation;
- Carnivorous diet and scavenging attitude;
- Mammal aggregation;
- Age: Juvenile mammals appear to be more susceptible to HPAIV infection, possibly due to their immature immune systems.

Environment:

- Proximity to farms with infected poultry or mammals.

Specific considerations about drivers for infection and adaptation to mammals of HPAI, e.g. environmental contamination, feed and proximity to bird outbreaks, were also provided for captive wild mammals, either farmed fur animals (mink, foxes, raccoon dogs), dairy cattle or from zoos and rehabilitation centres.

A specific concern is linked to the risk of influenza transmission by urban birds, in which the risk of influenza transmission to humans is generally considered lower than from domestic poultry or wild migratory birds, but it still poses some public health concerns, particularly because urban birds, such as pigeons, sparrows and crows, can act as reservoirs or intermediate hosts for AIVs. This may occur especially in urban settings where they may come into contact with infected migratory birds or poultry. The risk of direct transmission to humans remains low, but indirect risks, such as environmental contamination and potential cross-species transmission, highlight the need for continued monitoring and public health measures in urban areas (Shriner & Root, 2020).

Climatic factors:

- Climate change: Altered bird migratory patterns and habitat conditions due to extreme climatic conditions (droughts, heavy rains) can affect virus transmission dynamics.

Animal and public health measures:

- Effective monitoring of AI in susceptible animal species and early detection in humans are crucial to reduce spillover risk, only if followed by timely and appropriate measures to contain disease spread.
- Vaccination of poultry: Development and distribution of vaccines able to significantly reduce viral shedding for high-risk poultry populations can mitigate the risk of transmission to humans (as implemented in China against A(H7N9)). Moreover, vaccination against HPAI H5 in livestock in the USA is currently under trials for further consideration (USDA, 2024a, online-c).
- Ensuring front-line occupational groups are protected by raising awareness among their employers about the occupational safety and health measures to be applied to mitigate disease transmission and by limiting contact to susceptible animal populations during possible outbreaks may reduce spillover events.

Socio-economic factors:

- Health-care infrastructure: Countries with robust health-care systems can better manage and contain outbreaks when they occur.
- Public awareness: Education on safe handling of poultry and derived products, species at higher risk of infection and recognition of early symptoms can help reduce human exposure to AIV.

Therefore, the risk of AI becoming zoonotic is multifaceted, involving viral genetics, human–animal interactions, ecological factors and public health preparedness. Monitoring, surveillance and proactive measures are essential to mitigate the risk of a potential pandemic.

4.4 | Risk of zoonotic mutation of AIVs

In this section, the risk for viral mutation and adaptation to mammal species is assessed through an in-depth analysis of markers (mutations) of adaptation to mammals in AI genomic sequences available in public genetic databases along with associated metadata. The results are discussed from an epidemiological perspective. First, a set of shortlisted mutations is selected, and then, their presence in viral strains with their associated phenotypic traits linked to adaptation in mammals is analysed and discussed.

4.4.1 | Data collection and curation of avian influenza virus sequences

From all sequences of AI isolates available in the public sequence database GISAID from 1 January 2000 to 31 May 2024 (Puglia et al., 2024), we retrieved a total of 46 subtypes linked to spillover events in mammals. The analysed sequences were generated from viruses collected from January 2000 to May 2024 from avian and mammalian hosts including humans. The sequences originated from 1091 host species including 980 avian species and 111 mammalian species including humans, counted as downloaded from GISAID.[12] Bad quality sequences as well as sequences from H1 and H3 endemic strains circulating in swine, canine, equine species and human seasonal influenza viruses were then excluded to focus the analyses on AIVs. The final number of viruses after removing viruses for which a complete genome was not available and excluding viruses created in laboratory settings for experimental studies (e.g. reverse genetics viruses) was 27,482. This filtering process ensured that only field-derived viruses with full genome sequences were included in the scanning of zoonotic mutations.

For each downloaded isolate, the following was compiled along with the list of mutations related to the specific segment: isolate ID (virus identification), segment ID (segment identification), isolate name, subtype, clade, location (stratified by continent; state and specific location when available and divided into continent; state and when available), host, host class (avian or mammal), host family (mammalian families), collection date.

4.4.2 | Refining the selection of shortlisted mutations for zoonotic potential

The initial set of 592 mutations with known phenotypic effects related to mammalian adaptation, identified through a literature review (see Section 3.1.1) was narrowed down to 56 shortlisted mutations (Table 3) by applying the selection criteria outlined in Section 3.1.5. In brief, a mutation was considered a priority if it met one of the following four criteria: (1) a score of evidence of 4, (2) an evidence score of 3 along with identification in at least one pandemic representative strain, (3) mutations with an evidence score of 3 and considered relevant by at least one expert, even without pandemic determinant or (4) considered relevant by at least two experts, irrespective of the evidence score assigned.

A preliminary analysis of all the complete genome viral sequences was performed using these 56 initially shortlisted mutations and their frequencies in avian and mammalian hosts were calculated across different subtypes to further refine the shortlisted mutation list and increase the specificity of the analysis. This led to two distinct mutation subsets.

The first subset of 40 mutations (subset 1) was generated by removing mutations showing high frequency (> 80%) in avian viruses across different subtypes and geographic regions (Table 3). These highly prevalent mutations in avian virus sequences probably represent conserved regions of the viral genome and were excluded to increase the specificity of the zoonotic screening. Additionally, mutations with a frequency of 0% in AIVs collected from mammals were removed for practical reasons. The lack of field evidence of the occurrence of these mutations in AIV sequences from mammalian species used in this analysis highlights the need to further investigate their role in AIV adaptation to mammals. Out of subset 1 mutations, six mutations related to the trait 'increased virulence in mammals or general category in the absence of other information', which are indicated in Tables 3 and 4, were excluded from the trait-specific analysis; in total, 34 mutations out of subset 1 were included in the analysis.

The second subset (subset 2) of 13 mutations was generated by considering, among mutations in subset 1, only mutations with higher frequency in mammalian hosts compared with avian hosts (defined as a difference of > 1% in frequency between mammalian and avian samples) and a low frequency in avian species (< 30%), as these are suggestive of conferring an adaptive advantage in mammalian hosts (Table 3).

This multistep refinement of shortlisted mutations based on host frequency was necessary for practical reasons and analytical precision. Indeed, it cannot be ruled out that zoonotic mutations emerge and sustain within avian populations, as was the case for the PB2 627K mutation for viruses of the Qinghai Lake outbreak in 2005 and descendants that later spread to Europe and Africa. However, initial analyses using all 56 mutations lacked specificity (data not shown), as some of them were present with a high frequency in subtypes not known to cause zoonotic events. This suggests that further investigations are needed to assess the impact of some of the 56 mutations selected according to the criteria described in Section 3.1.4.

The 40 selected mutations (subset 1) are associated with six distinct phenotypic traits, as mentioned in Section 3.1.1. Five of these six traits associated with 34 out of 40 mutations represent the adaptive changes that AIVs must acquire to potentially cause a pandemic:

1. Increasing mammalian specificity of virus attachment to receptors.
2. Increasing HA stability in the mammalian environment.
3. Disruption of the second sialic acid-binding site (2SBS) in neuraminidase.
4. Enhanced activity of viral polymerases in mammalian hosts.
5. Evasion of innate immunity and counteraction of mammalian restriction factors.

[12]This number of species may be an overestimation due to a lack of standardisation of data about host species definition in GISAID, when duplicates or non-univocal species definition may be reported. The number of mammal species ever reported to the WOAH affected by AI is indicated in Section 4.1.

A total of 34 mutations from subset 1 and all the 13 mutations of subset 2 fall within these five phenotypic categories (Table 3). Only the mutations associated with these five traits will be described in detail in Sections 4.4.3.1–4.4.3.5. The sixth trait (increased virulence in mammals or general category in the absence of other information) includes mutations that, according to current literature, have effects that cannot be clearly attributed to any of the specific categories mentioned above.

TABLE 3 List of 56 mutations selected based on their frequency in mammals and avian species (% indicate the frequency of occurrence in avian or mammalian species).

			Criteria for prioritisation	
Mutation	**Mammals**	**Avian**	**Subset 1 ($n = 40$ mutations) based on < 80% in avian species; > 0% in mammalian species**	**Subset 2 ($n = 13$ mutations) based on < 30% in avian species; % mammal – % avian > 1%**
HA:156A	87.70%	74.52%	✓	
HA:156V	0.37%	0.68%	✓	
HA:186D,221D	0.05%	0.00%	✓	
HA:186V	0.88%	1.48%	✓	
HA:208T	1.76%	13.42%	✓[a]	
HA:221D	0.32%	0.10%	✓	
HA:222L	56.01%	13.92%	✓	✓
HA:224S	0.37%	1.87%	✓	
HA:307K	94.52%	83.12%		
MP1:215A	100.00%	99.99%		
MP1:242R	0.00%	0.02%		
MP1:30D	99.72%	99.94%		
MP1:95K	63.76%	22.15%	✓	✓
NA:399R	0.32%	1.03%	✓	
NA:432E	1.67%	22.66%	✓	
NP:100I	0.32%	0.00%	✓	
NP:100V	0.23%	0.01%	✓	
NP:283P	0.09%	0.01%	✓	
NP:313V	0.19%	0.01%	✓	
NP:313Y	0.09%	0.02%	✓	
NP:52H	14.34%	15.35%	✓	
NP:52N	66.17%	19.52%	✓	✓
NS1:106M	38.24%	85.93%		
NS1:42S	99.35%	90.30%		
PA:186S	0.23%	0.46%	✓[a]	
PA:336M	2.37%	1.50%	✓[a]	
PA:356R	59.49%	11.95%	✓	✓
PA:36T	0.00%	0.23%		
PA:383D	99.77%	99.49%		
PA:550L	99.68%	99.02%		
PA:552S	0.14%	0.03%	✓	
PA:85I	0.42%	0.09%	✓	
PA:97I	0.32%	0.08%	✓	
PB1-F2:66S	19.35%	48.97%	✓	
PB1:3V	99.54%	99.19%		
PB1:622G	99.81%	99.34%		
PB2:158E	99.91%	99.80%		
PB2:199S	0.37%	0.23%	✓[a]	
PB2:271A	1.25%	0.14%	✓	✓
PB2:292V	55.73%	24.36%	✓	✓
PB2:389R	38.61%	82.94%		

TABLE 3 (Continued)

Mutation	Mammals	Avian	Criteria for prioritisation: Subset 1 ($n = 40$ mutations) based on < 80% in avian species; > 0% in mammalian species	Criteria for prioritisation: Subset 2 ($n = 13$ mutations) based on < 30% in avian species; % mammal – % avian > 1%
PB2:526R	9.33%	2.23%	✓	✓
PB2:559T	80.46%	86.95%		
PB2:588I	0.32%	1.49%	✓	
PB2:588V	26.45%	11.07%	✓	✓
PB2:591K	1.95%	0.28%	✓	✓
PB2:591R	0.79%	0.04%	✓	
PB2:627K	46.87%	0.95%	✓	✓
PB2:627V	3.06%	0.88%	✓	✓
PB2:631L	8.63%	0.46%	✓	✓
PB2:701N	5.15%	0.08%	✓	✓
PB2:702R	4.87%	7.27%	✓	
PB2:740N	0.28%	0.34%	✓[a]	
PB2:76T	99.16%	95.21%		
PB2:89V,309D	99.49%	98.16%		
PB2:9N	1.25%	0.60%	✓[a]	

[a]These mutations were not included in the trait-specific analyses. The total number of mutations included in the analysis is 34 from subset 1 and 13 from subset 2.

TABLE 4 Phenotypic trait (blue), mutation effect, score of evidence and output from expert assessment of the shortlisted mutations (subset 1 with all 40 mutations, the 13 mutations of subset 2 are also indicated). HA positions are provided using H5 numbering (in parentheses H3 numbering).

Mutation[a]	Effect	Score of evidence	Selected by no. experts	13 shortlisted mutations
Trait: Increases mammalian specificity of virus attachment to receptor (receptor preference)				
HA:156A (160A)	Increased virus binding to α2,6, increased transmission in guinea pigs	4	2	
HA:156V (160V)	Observed in seal samples; decreased virulence in mice and increased affinity for the human-type receptor	3	3	
HA:186D,221D (190D,225D)	Switch in the receptor specificity from avian-type to human-type receptor	2	6	
HA:186V (190V)	Enhances binding affinity to mammalian cells and replication in mammalian cells; enhanced replication in mice	4	4	
HA:221D (225D)	Increased virus binding to α2,6; increased virus binding to α2,6	3	3	
HA:222L (226L)	Increased virus binding to α2,6; transmitted via aerosol among guinea pigs; enhanced replication in mammalian cells and ferrets, enhanced contact transmission in ferrets; Loss of binding to α2,3	4	4	✓
HA:224S (228S)	Increased binding to α2,6; increased viral replication in mammalian cells and virulence in mice; observed in human isolate; increase in the ability of the virus to infect mammals; decreased virus binding to α2,3	4	4	
Trait: Increases HA stability in mammal's environment (decreased pH of fusion, increased thermal stability)				
HA:222L (226L)	Increased acid and thermal stability	4	4	✓
Trait: Disruption of the second sialic acid binding site (2SBS) in neuraminidase				
NA:399R	Mutation of 2SBS that are detrimental to the cleavage of sialosides linked to fetuin or transferrin; observed in seals	3	2	
NA:432E[b]	Decreased cleavage of fetuin-containing α2,3-linked sialic acids (SIAs) but not that of monovalent substrates or of transferrin containing only α2,6-linked SIAs	3	2	
Trait: Increased activity of the viral polymerases in mammalian hosts				
PA:356R	Human host marker; Increase polymerase activity and enhanced replication in a mammalian cell line, increased virulence in mice	4	1	✓

(Continues)

TABLE 4 (Continued)

Mutation[a]	Effect	Score of evidence	Selected by no. experts	13 shortlisted mutations
PA:552S	Enhanced viral RNA-dependent RNA polymerase (vRdRp) activity and viral replication in vitro	2	2	
PA:85I	Enables guanine-rich sequence binding factor (GRSF1) to enhance the cytosolic accumulation and translation of a subset of viral mRNAs; enhanced replication in human A549 cells	2	2	
PA:97I	Increased polymerase activity in mammalian cell line and enhanced replication and virulence in mice; increased polymerase activity in mammalian	4	1	
PB1-F2:66S	Increased virulence, replication efficiency and antiviral response in mice	3	3	
PB2:271A	Increase polymerase activity in the presence of avian acidic nuclear phosphoprotein 32 (ANP32) proteins; increase mortality and airborne transmission in ferrets; increased polymerase activity in avian and mammalian cell line	4	3	✓
PB2:292V	Observed in H3N8 samples from humans and birds; increased polymerase activity in a mammalian cell line, increased virulence in mice	4	1	✓
PB2:526R	Increased polymerase activity in mammalian cell line and virulence in mice	3	3	✓
PB2:588I	Enhances 2009 H1N1 pandemic influenza virus virulence by increasing viral replication and exacerbating PB2 inhibition of beta-interferon expression	2	2	
PB2:588V	Increased polymerase activity and replication in mammalian and avian cell lines, increased virulence in mice	4	2	✓
PB2:591K	Increased polymerase activity in mammalian and avian cell line, increased replication in a mammalian cell line, increased virulence in mice; observed in human isolates	3	2	✓
PB2:591R	Enhanced mammalian ANP32 adaptation (non-biased)	3	2	
PB2:627K	Observed in human isolate; increased polymerase activity and replication in mammalian cell line, increased virulence in mice and ferrets; contributes to airborne transmission of influenza A viruses (IAVs) in ferrets and contact transmission in guinea pigs; mammalian ANP32-specific adaptation (ANP32B biased)	4	4	✓
PB2:627V	Observed in human isolate; mammalian ANP32-specific adaptation (ANP32B biased); observed in human samples in Shenzhen; increased polymerase activity and replication in mammalian cell lines, increased virulence in mice	3	3	✓
PB2:631L	Most dominant mutation in mouse-adapted virus that strongly upregulated viral polymerase activity and played a critical role in the enhancement of virus replication and disease severity in mice; observed in dairy cattle USA 2024	3	2	✓
PB2:701N	Increased polymerase activity, enhanced replication efficiency, increased virulence and contact transmission in guinea pigs; increased polymerase activity in mammalian cell line; increased viral replication and virulence in mice; mammalian ANP32-specific adaptation (non-biased); observed in seals	4	3	✓
PB2:702R	Observed in human isolate; increased polymerase activity in human and avian cells	3	2	
Trait: Evasion of innate immunity and counteraction of mammalian restriction factors				
MP1:95K	Resistant to TRIM21, increases replication and pathogenicity in mice	4	1	✓
NP:100I	Resistance against Myxovirus resistance protein A (MxA)	4	6	
NP:100V	Resistance against MxA	4	5	
NP:283P	Resistance against MxA	3	4	
NP:313V	Role in Butyrophilin Subfamily 3 Member A3 (BTN3A3) evasion	4	5	
NP:313Y	Role in BTN3A3 evasion	3	6	

TABLE 4 (Continued)

Mutation[a]	Effect	Score of evidence	Selected by no. experts	13 shortlisted mutations
NP:52H	Role in BTN3A3 evasion	3	3	
NP:52N	Significant mutation observed also in H5N1; role in BTN3A3 evasion	4	5	✓
Trait: increased virulence in mammals or general category in the absence of other information[c]				
PA:336M	Significantly enhanced pathogenicity in a mouse model	2	2	
PB2:199S	Increased virulence in mice	2	2	
PB2:9N	Observed in domestic poultry, Uganda; increased virulence in mice	3	2	
HA:208T	Increased the viral replication in avian and mammalian cells; enhance viral replication in mice	3	2	
PA:186S	Enables GRSF1 to enhance the cytosolic accumulation and translation of a subset of viral mRNAs	2	2	
PB2:740N	Increase polymerase activity in the presence of avian ANP32 proteins, in human and avian cells	2	2	

[a]To increase the sensitivity of the method, a lower threshold was set for considering a mutation as relevant (in criteria in step 4 and expert opinion, Section 3.1.5). For this reason, some mutations that were shortlisted among the 40 (e.g. 9N and 199S in PB2 and 208T in HA that were flagged mainly due to their presence in previous pandemic viruses) may be less relevant for assessing the zoonotic potential.

[b]This mutation (432E in NA) was shortlisted due to the use of a suboptimal reference for assigning mutations that were observed during the 1918 pandemic in the 'pandemic determinant' tab (see Section 3.1.3), and potentially less relevant.

[c]This trait and the included mutations (in grey) were not considered in the trait-specific analyses. The total number of mutations included in the analysis are 34 from subset 1 and 13 from subset 2.

4.4.3 | Analysis of mutations and traits in AIV strains

In this section, the distributions of mutations of interest with each related phenotypic trait across the downloaded viruses' sequences are discussed in terms of frequency of mutations in subtypes, clades and geographical origin. Possible limitations, biases and uncertainties linked to this analysis can be found in Section 4.8. Bioinformatics tools (e.g. FluMut; https://izsvenezie-virology.github.io/FluMut/) can also be used for the rapid identification of critical mutations on generated sequences.

4.4.3.1 | *Mutations associated with increased mammalian specificity of virus attachment to receptor (receptor preference)*

A critical evolutionary step for AIVs to gain pandemic potential involves mutations in the HA RBD that shift binding preference from α2,3-linked to α2,6-linked sialic acids. Influenza A viruses (IAVs) infect host cells by binding the haemagglutinin (HA) protein to sialic acid receptors on the cell surface. Human influenza virus HAs primarily exhibit a preference for binding to α2,6-linked sialic acid (α2,6 receptor), whereas those of avian origin display a higher binding affinity for α2,3-linked sialic acid (Matrosovich et al., 1997, 2000). The binding patterns and the distribution of sialic acid in human tissues provide an explanation why AIVs usually cannot easily infect humans. In humans, α2,6 receptors are mainly expressed on cells of the ciliated epithelium along the upper respiratory tract (URT), while α2,3 receptors are primarily found in the lower respiratory tract (Shinya et al., 2006) and the human eye (conjunctiva) (Chan et al., 2010). Then, for efficient respiratory transmission in humans, influenza viruses need to replicate in the URT, where α2,6-linked sialic acid receptors are predominant.

Mutations associated with the acquisition of an increased affinity towards α2,6-linked sialic acid receptors allow AIVs to increase adaptation to human respiratory tract characteristics, facilitating transmission and increasing zoonotic potential.

Seven of the subset 1 shortlisted mutations are associated with this specific trait, while only one of them, namely the HA:222L (226L according to H3 numbering), falls within the subset of 13 shortlisted mutations.

With the exception of mutation HA:156A, which is present in a multitude of viruses across different subtypes (32 out of 46 subtypes here analysed) with a high frequency, ranging between 27% and 100%, and has been identified in both avian and mammalian species, the mutations associated with this trait (i.e. increased affinity towards α2,6-linked sialic acid receptors) have been identified only sporadically among the various subtypes analysed in this study. When present, they have appeared primarily in a few AI subtypes. This is the case for mutation **HA:222L** (226L according to H3 numbering), which was detected in 10 different subtypes, although in eight of these, it appeared at low frequencies, always below 9%. However, in two specific subtypes, A(H7N9) and A(H9N2), it was found at frequencies exceeding 80% (Figures 10–16). A(H7N9) viruses characterised by the presence of this mutation were all sequenced in Asia in both avian and mammalian species (human, mouse and swine), starting from 2013, with the most recent A(H7N9) carrying this mutation being identified in 2018. Regarding A(H9N2), viruses carrying this mutation have been identified on the Asian and African continents, primarily in poultry but also in some mammalian species, including mink, swine, a cat and humans.

Among the additional subtypes in which this high-priority mutation has been detected are other viruses of the H7 subtype (H7N2 and H7N6) identified in birds in Asia between 2014 and 2017. This mutation has also been found in H5 HPAIVs of the Gs/GD/96 lineage, specifically in A(H5N6) viruses of clade 2.3.4.4h identified in avian species in Asia between 2017 and 2019, as well as in one instance (A/canine/China/GX30/2023) within clade 2.3.4.4b. Additionally, two A(H5N1) viruses belonging to clade 2.2.1.2, which were identified in avian species in Egypt in 2017, were also found to carry this mutation. The mutation has also been sporadically identified in H6 subtype viruses (H6N6 and H6N2) and in an A(H10N3) virus responsible for a human case.

The other mutation associated with enhanced binding affinity for the human-type sialic acid receptor, found at high frequency (> 8.4%) in A(H9N2) subtype viruses, is the **HA:186V** substitution. It has been identified in A(H9N2) virus sequences from Asia and Africa, primarily in poultry and, in a few cases, also in mammals, including humans. This mutation has also been found at low frequency in other subtypes, particularly in H5N2 viruses (approximately 6% of sequences belonging to this subtype) and primarily belonging to the American lineage H5N2 viruses. It has also been detected in H6N1 and H6N2 viruses (approximately 4% in each subtype) and at a very low frequency in H5N6 viruses of clade 2.3.4.4h (specifically, two viruses identified in poultry).

Interestingly, another relevant adaptive mutation, **HA:224S** (228S according to H3 numbering), which has been shown to play a role in switching binding specificity, was rarely detected across the AI subtypes analysed. However, it was present in all H13 subtype sequences included in this study, specifically in the H13N2, H13N6, H13N8 and H13N9 subtypes. In most cases, these viruses were identified in avian species from the Laridae family, spanning geographic regions from the Americas to Eurasia. Additionally, subtypes in which the mutation was observed in > 4% were H10N3 viruses in Asia observed at a frequency of 18%, primarily in poultry sampled between 2019 and 2022, as well as in two human cases identified in 2021 and H6N1 viruses in Asia at a frequency of > 4%, mainly in poultry, with one occurrence in a human case.

Only one H4N6 virus exhibited the **226L** and **228G** (H3 numbering) combination previously identified in pandemic viruses (H2 and H3 HAs). Specifically, this virus was identified in a pig in the USA in 2015 (A/swine/Missouri/A01727926/2015).

In relation to the 186D and 221D (E190D and G225D) mutations, which have been demonstrated to cause a switch in binding specificity in the H1 subtype and have been identified in previous H1 pandemic strains, none of the AIVs available for this analysis showed their presence in the same virus. However, despite a very low frequency (always less than 1%), one of the two mutations, the HA:221D mutation (225D according to H3 numbering), has been identified in certain subtypes including H9N2 viruses from poultry in Asia and Africa, H7N9 viruses identified in humans in Asia, and H3N8 viruses identified in central Asia from avian species.

Another mutation, besides 156A, that is found in a range of different AI subtypes (13 subtypes) and across distinct geographic regions is mutation **156V** (corresponding to the A160V substitution according to H3 numbering). The subtypes in which it appears with the highest frequency are A(H7N1), present in both European and American viruses, all identified in avian species, and American lineage H5N2, also predominantly of avian origin, with the exception of two sequences from H5N2 viruses identified in Mexico in mammals (swine).

The temporal trend of these mutations varies significantly depending on the specific mutation (Figure 17). For example, it is interesting to note that the widespread HA:156A has markedly increased in frequency since 2021 as a result of its presence in 98.5% of H5 HPAIVs belonging to clade 2.3.4.4b, which has been responsible for the ongoing panzootic. The frequency trend of this mutation does not align with the trend in human cases, and given its high prevalence across multiple subtypes, it can be hypothesised that this mutation is well tolerated in the avian virus genome and its zoonotic impact remains to be further defined. Previous studies have shown that amino acid substitutions T154D or T156A that caused the loss of the 156–158 (NST) glycosylation site in the HA resulted in increased binding to α2,6-sialic acid without a loss of binding to α2,3-sialic acid. This might explain the high frequency of 156A in the analysed sequences, as this mutation may not cause a substantial change in the receptor-binding affinity, unless it is present in combination with other mutations in the RBS (i.e. 222L) (Linster et al., 2014; Tharakaraman et al., 2013). Combinations of mutations have, however, not been included in the analysis (see Section 4.8) unless they have been studied only in combination and not individually.

Conversely, it is interesting that the high-priority 222L mutation (along with other mutations) experienced an increase between 2012 and 2017, coinciding with the rise in human cases associated with the H7N9 subtype (Figures 8 and D.5, D.6 in Annex D). It should be noted in particular that 51% of the viruses identified with this mutation in this time interval belong to the H7N9 subtype, and all sequences of this viral subtype with this mutation have been identified in China except for one sequence obtained from a human case reported in Canada in a patient returning from China (Skowronski et al., 2016). However, proving the association between genetic data and human case numbers remains challenging due to the lack of published sequences for many human cases, as well as difficulties in correlating these data with the overall number of poultry infection cases.

4.4.3.2 | *Mutations associated with increased HA stability in mammal's environment (decreased pH of fusion, increased thermal stability)*

Over the past decade, there has been a growing recognition of the significance of HA stability in influencing pandemic potential. Indeed, recent research has underscored the importance of HA protein stabilisation in enabling human adaptation and facilitating airborne transmission in experimental ferret models. For efficient human-to-human transmission to occur, HA needs to be stable and triggered only at more acidic pH so it survives the acidic microenvironment of airborne particles and mammalian respiratory secretions (Imai et al., 2012). Amino acid substitutions in over 50 HA residues across H1, H2, H3,

H5 and H7 subtypes that have the potential to alter HA stability have been identified (Russell et al., 2018). It needs to be noted that the analysis performed here only partially covered the residues that have been previously described to alter HA stability (see Section 4.8), and therefore, other mutations may also be relevant and need to be monitored.

The only mutation in the shortlisted subsets that falls into this category is the **222L** mutation. For details on its geographical and temporal distribution across viral subtypes, please refer to the previous Section 4.4.3.1. Indeed, this mutation has been demonstrated in the literature to be associated with both increased binding affinity and enhanced HA stability traits, as shown in Table 3.

4.4.3.3 | *Mutations associated with disruption of the second sialic acid binding site (2SBS) in neuraminidase*

As another major viral surface protein alongside HA, NA possesses sialidase activity and is responsible for cleaving SA from the cell surface to facilitate the release of the progeny virus particle (Schrauwen et al., 2014). There is evidence that maintaining a functional balance between the SA binding ability of HA and the sialidase activity of NA is a crucial factor determining the host fitness of influenza viruses.

Among the subset 1 shortlisted mutations, there are two mutations in the NA target that affect the binding and cleavage of receptors, thereby enabling more efficient interactions with mammalian hosts. One of these, NA:432E, is detected at very high frequencies (≥ 99%) in 14 distinct subtypes globally and at low frequencies in six additional subtypes and is likely to be less relevant for its zoonotic potential (see Table in Section 4.4.2). The other NA mutation, NA:399R, appears much less frequently. It was identified in only five subtypes and at a very low frequency, except for the H9N2 subtype, where the mutation frequency was 7.5%. In particular, the mutation is present in viruses of subtype H9N2 identified in Asia in birds except for one human case identified in India in 2019.

Overall, this phenotypic trait was only identified in 11 of 46 subtypes identified in mammals. Interestingly, however, this trait was found in all 15 H3N8 sequences identified in mammals (pinnipeds and humans), and in four H10N8 sequences of human origin from 2013 to 2014, as well as in all the sequences belonging to the H5N5 (7/7 sequences) and H5N8 (6/6 sequences) subtypes, clade 2.3.4.4b, identified in wild mammals in Europe and North America.

The bioinformatics analyses performed here did not include characterisation of the stalk region of the neuraminidase (NA) viral surface protein. A short stalk length in the NA protein has been described as an additional factor limiting the mammalian transmission of AIVs. However, due to variations in the length and position of the stalk region across different NA genes and subtypes, it was not feasible to consistently monitor this feature across the comprehensive data set of sequences used here. Any NA stalk deletion should signal a potential adaptive change of the neuraminidase and therefore should also be closely monitored.

Regarding the temporal trend of these mutations, a peak was observed for the NA:432 mutation between 2020 and 2021. This peak corresponds to the presence of the mutation in N8 subtype viruses from 2020 to 2021, specifically in H5N8 clade 2.3.4.4b viruses and in H3N8 subtype viruses identified primarily in poultry in Asia. However, this peak was not associated with an increase in the number of human cases.

4.4.3.4 | *Mutations associated with increased activity of the viral polymerases in mammalian hosts*

The RNA-dependent RNA polymerase (RdRp) of influenza viruses consists of polymerase protein basic 1 (PB1), polymerase protein basic 2 (PB2) and polymerase acidic protein (PA). Mutations in the polymerase proteins are important determinants of virus host range. They have been shown to play a critical role in the pathogenicity and adaptation of avian viruses to mammalian hosts, markedly raising the RdRp activity in mammalian cells (Suttie et al., 2019). The significance of these amino acids in the virus adaptation to mammals is emphasised by the fact that 17 from subset 1 and 10 from subset 2 shortlisted mutations are associated with this trait.

Considering the subset 1 shortlisted mutations, all the subtypes possess mutations associated with this increased activity of the viral polymerases in mammalian hosts, and for 39 different subtypes, more than 50% of the viruses possess at least one of them. The most selective analysis performed on the 13 mutations shows that, although this trait has been identified in almost all subtypes (43/46), it has been commonly observed (> 50% of the viruses) in just a few of them (H9N2, H7N9, H5N8 and H5N6) (Figure 15). Of note, among the HPAI H5 viruses of the Gs/GD/96 lineage, this trait is frequently identified in viruses of clades 2.1.3.x, 2.2.x, 2.3.2.1a, 2.3.4.4.x and 7.x (where x is for subclades originating from that clade). In particular, within the currently widespread clade 2.3.4.4, Subclades 2.3.4.4b, 2.3.4.4c, 2.3.4.4e, 2.3.4.4g and 2.3.4.4h are the ones showing the highest frequency of the 17/40 mutations associated with this trait, while considering the 10/13 associated trait mutations the high trait frequency (> 60%) was observed only for Subclades 2.3.4.4e, 2.3.4.4g and 2.3.4.4.h.

In detail, five of the mutations associated with increased mammalian specificity of viral polymerase (PA:356R, PB1-F2:66S, PB2:292V, PB2:588V, PB2:702R) have been identified in half or more than half of the analysed subtypes with a relatively high frequency (> 60%) in some of them. These five mutations are distributed in viruses from all continents, except Oceania (Figures D.2–D.4, Annex D), where PA:356R, PB2:588V and PB2:702R have not been detected. This suggests that some of the mutations associated with mammalian adaptation in the polymerase proteins can be promptly acquired by AIVs without affecting their capability to replicate and be efficiently transmitted among avian hosts. On the other hand, the other 12 mutations associated with this trait have been detected in at least eight different subtypes but with a relatively low frequency in most of them, with few exceptions: PB2:526R shows a frequency of 18% and 12% in the H3N1 and H7N9 subtypes, PB2:627K has a frequency of 42% in the H7N9 subtype, PB2:271A shows a frequency of 11% in the H4N1 subtype.

All the mutations associated with this specific trait have been identified in Asia and most of them (16/17) are also in Europe and North America.

Some of the 17 mutations show an increase in their detection frequency in certain years. Starting from 2013, up to 2017–2022 we observed a rise in the frequency of PA:356R, PB2:292V, PB2:588V and PB2:627K. Of note, all these four mutations were present simultaneously in viruses belonging to the H7N9 ($N = 219$), H9N2 ($N = 7$), H3N8 ($N = 6$) and H10N8 ($N = 2$) collected in China from human (H7N9, H9N2, H3N8, H10N8) and avian (H9N2 and H10N8) hosts. This rise in the frequency detection of these mutations overlaps with a rise in human cases in China, mainly associated with the H7N9 subtype (Figure 17).

Recently (2023–2024), three mutations have shown a steep (PB2:631L) or slight rise (PB2:271A and PB2:627K) in their frequency (Figure 17). The mutation PB2:631L is strictly associated with the H5N1 outbreaks reported in cattle in the USA and complements experimental evidence that this mutation enhances polymerase activity in human cells (Gu et al., 2024) and increases the virulence of avian H10N7 in a mammalian host (Zhang et al., 2017). Also, the rise of mutations PB2:271A and PB2:627K can be mainly ascribed to the H5 viruses of the Gs/GD/96 lineage and specifically to clades 2.3.4.4b or 2.3.2.1c detected in avian and mammalian species from Europe, Asia and North and South America, indicating a sporadic and independent emergence of these markers.

Overall, the analyses of each single strain indicated that 22,145 out of 27,480 (80.6%) contain at least one of the molecular markers of mammalian adaptation in the polymerase proteins, with a maximum of six mutations present in a single viral strain and five mutations occurring in 55 viruses. These viruses were collected from human (one H10N3 from 2024; three H3N8 from 2022, 34 H7N9 from 2014 to 2017 and five H9N2 from 2018 to 2021), non-human mammalian (one H5N1 from mink) and avian (H10N8, H3N8, H5N1, H5N2, H7N9 and H9N2) hosts, with the vast majority of these viruses originating from China.

4.4.3.5 | *Mutations associated with the evasion of innate immunity and counteraction of mammalian restriction factors*

Overcoming innate immunity is an essential step for AIVs to infect and transmit efficiently in a new host population. Recent studies have demonstrated that antiviral factors, like MxA, butyrophilin subfamily 3 member A3 (BTN3A3) and tripartite motif-containing protein 21 (TRIM21), represent an important barrier for the replication of avian IAVs in the human host (Götz et al., 2016; Lin et al., 2023; Pinto et al., 2023). Adaptive mutations that counteract cellular restriction factors of the innate immune system represent one of the risk factors for the zoonotic potential of avian IAVs.

Eight of the subset 1 shortlisted mutations are associated with this specific trait, while only two of them, namely NP:52N and MP1:95K, fall within the subset of 13 shortlisted mutations.

Among the analysed subtypes, 41 possess at least one of the eight mutations associated with the evasion of mammalian restricting factors. However, only in the H5N2, H5N6, H7N9, H9N2 and H13Nx subtypes this trait has been commonly observed (60% of the viruses). Specifically, mutations NP:52N/H associated with BTN3A3 evasion were identified in all or almost all the viruses of the H13Nx, H7N9 and H9N2 subtypes and sequences with this substitution were detected in all continents. An increase in the frequency of these two substitutions has been observed between 2012 and 2019 and in 2024, with the last peak mainly due to clade 2.3.4.4b viruses, including those responsible for the outbreaks in dairy cattle in the USA. Mutation MP1:95K, associated with resistance to TRIM21, is present in the vast majority of viruses of the H7N9 and H9N2 subtypes and in 50% and 30% of the H5N2 and H10N8 subtypes, respectively. Except for Oceania, this mutation has also been identified in all continents, with a peak of detections between 2013 and 2019 (Figure 17). Of note, 5203 analysed viruses contained both substitutions in position 52 of the NP and 95 of the MP1, and more than 90% of them belonged to the H9N2, H7N9 and H3N8, three subtypes associated with several human cases.

The mutations associated with resistance against MxA have been very sporadic in the different AI virus subtypes, but among the HPAI H5 viruses of the Gs/GD/96 lineage, this trait has been frequently (> 60%) identified in viruses belonging to 13 different clades (Figure 13). Mutations associated with resistance to TRIM21 are most frequently detected in clades 2.1.3, 2.1.3.2.x, 2.2.1.2 and 2.3.4.4c and mutations linked to BTN3A3 evasion in clades 2.3.4.4e, 2.3.4.4, 2.3.3, 2.3.2.1b, 2.3.2.1.c (Figure 16).

4.4.4 | Analysis of shortlisted mutations across multiple genes in AIV subtypes

The combined analysis of the distribution of the 34 shortlisted mutations and their associated respective traits shows that, among the 46 AI subtypes with available whole genome data, the maximum number of mutations and traits identified in a single strain was 11 (out of 34) and 5 (out of 5), respectively. Only rarely have multiple shortlisted mutations (≥ 8) and associated traits (at least 3) been found together in the same virus. The threshold of three traits and eight mutations was chosen arbitrarily as a heuristic approach to enhance specificity in the analysis of viral genomes retrieved from the GISAID database, focusing on viruses with a higher number of mutations (8–11) and associated traits (3–5).

Among the 27,480 viruses studied, only 551 had at least eight of the 34 mutations (subset 1) in their genome and at least three traits associated with them. It is also interesting to note that the viruses with this high number of mutations and traits are restricted to just a few subtypes. Specifically, among these 551 sequences, 60% are A(H7N9), 36% A(H5N6) and 34% are A(H9N2), followed by 5% A(H3N8), and a handful of viruses belonging to the A(H5N1) (4%), A(H10N3) (2%) and A(H10N8)

(2%) subtypes. Most of these viruses were identified in Asia, primarily in poultry but also in mammals, with one exception, an A(H5N1) (LPAI, non-Gs/GD) identified in wild birds in Europe in 2023.

In the past 3 years (2021–2024), there were 38 viruses with ≥ 8 mutations and three to five traits, all belonging to subtypes H9N2 (74%) and H3N8 (21%), except for two viruses that fell under subtypes H10N3 and H5N1 (Eurasian non-Gs/GD H5 lineage), respectively. They were all detected in Asia in either poultry or humans except for the aforementioned H5N1 virus, which was identified in northern Europe.

In EU/EEA, between 2021 and 2024, there was only one virus that had ≥ 8 mutations that were associated with three traits; it was of the H5N1 subtype (LPAI, non-Gs/GD). There were, however, a number of viruses, mainly belonging to subtypes H5Nx (H5N1, H5N2, H5N5 and H5N8) all of which belonged to clade 2.3.4.4b (1677), that had a lower number of mutations (< 8) associated with three (1667 viruses) or four (10 viruses) traits. A handful of other subtypes also had a lower number of mutations associated with three or four traits: H13Nx (7), H3N8 (6), H6Nx (3) and H7N7 (1).

In Figure 9, all subtypes are displayed according to the percentage of sequences that accumulated each number of traits, highlighting those with three to five traits, irrespective of the number of mutations. Multiple traits are probably required for an AI virus to acquire pandemic potential and the accumulation of traits increases the risk of a virus becoming a pandemic threat. Noteworthy, overall, only H9N2 viruses (144) accumulated five traits; 51/144 were recently detected, between 2021 and 2024. In total, 124 recent viruses (2021–2024) had four traits (irrespective of the number of accumulated mutations), out of which 105 were H9N2 and the remaining 19 belonged to subtypes H5N5 (8), H5N8 (4), H5N1 (2), H3N8 (2), H7N7 (1), H10N3 (1) and H13N8 (1) (Figure 9).

Interestingly, out of a total of 46 AIV subtypes, those that have been reported to have caused most human cases so far – i.e. H7N9, H9N2, H5N1, H5N6, H7N7 – but also subtypes with rarer occurrence in human cases – e.g. H5N8, H10N8, H10N3, H3N8 – are among the subtypes that have also been identified through the mutation analysis as subtypes that tend to have a higher number of adaptive traits (Figure 9). Moreover, 91% of sequences from human cases (1354/1485) carried mutations associated with three (11%), four (80%) or five (< 1%) traits.

Some recent 2021–2024 H5N1 viruses from clades 2.3.2.1a (1), 2.3.2.1c (90) and 2.3.4.4b (2455) were associated with three traits and two viruses of clade 2.3.4.4.b viruses with four traits. In total, 555 and four clade 2.3.4.4.b H5N8 viruses have three and four traits, respectively. A few H5N6 viruses had mutations associated with three traits: clades 2.3.4.4b (8) and 2.3.4.4h (3). Out of these recent clade 2.3.4.4b H5Nx viruses, a proportion of the H5N1 (1298/2455), H5N8 (335/555) and all H5N5 (31/31) clade 2.3.4.4b viruses were from Europe.

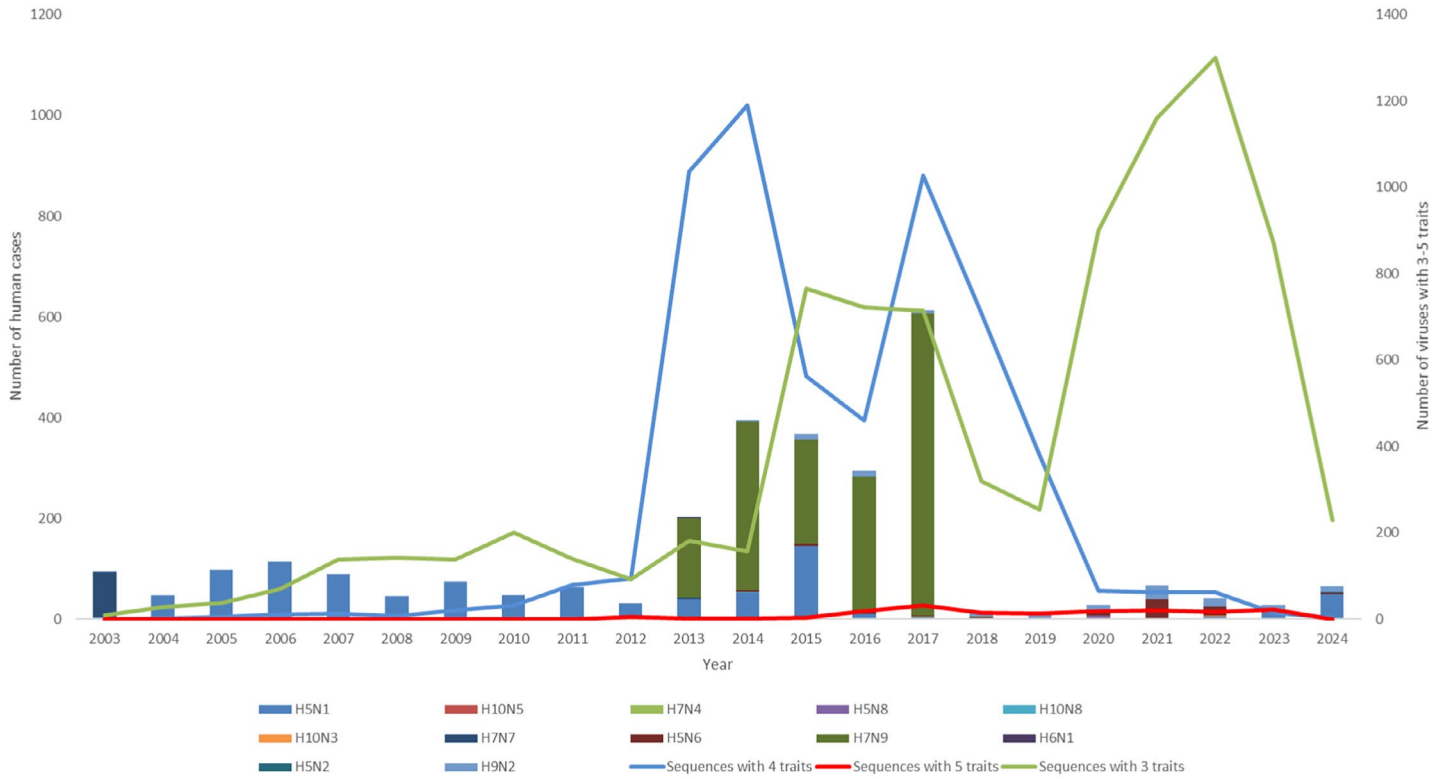

FIGURE 8 Timeline of human cases by virus subtype and the total number of sequences (avian and mammalian) with a higher number of accumulated traits (3–5). The left y-axis is the number of human cases; the right y-axis is the number of virus sequences with mutations affecting three to five traits.

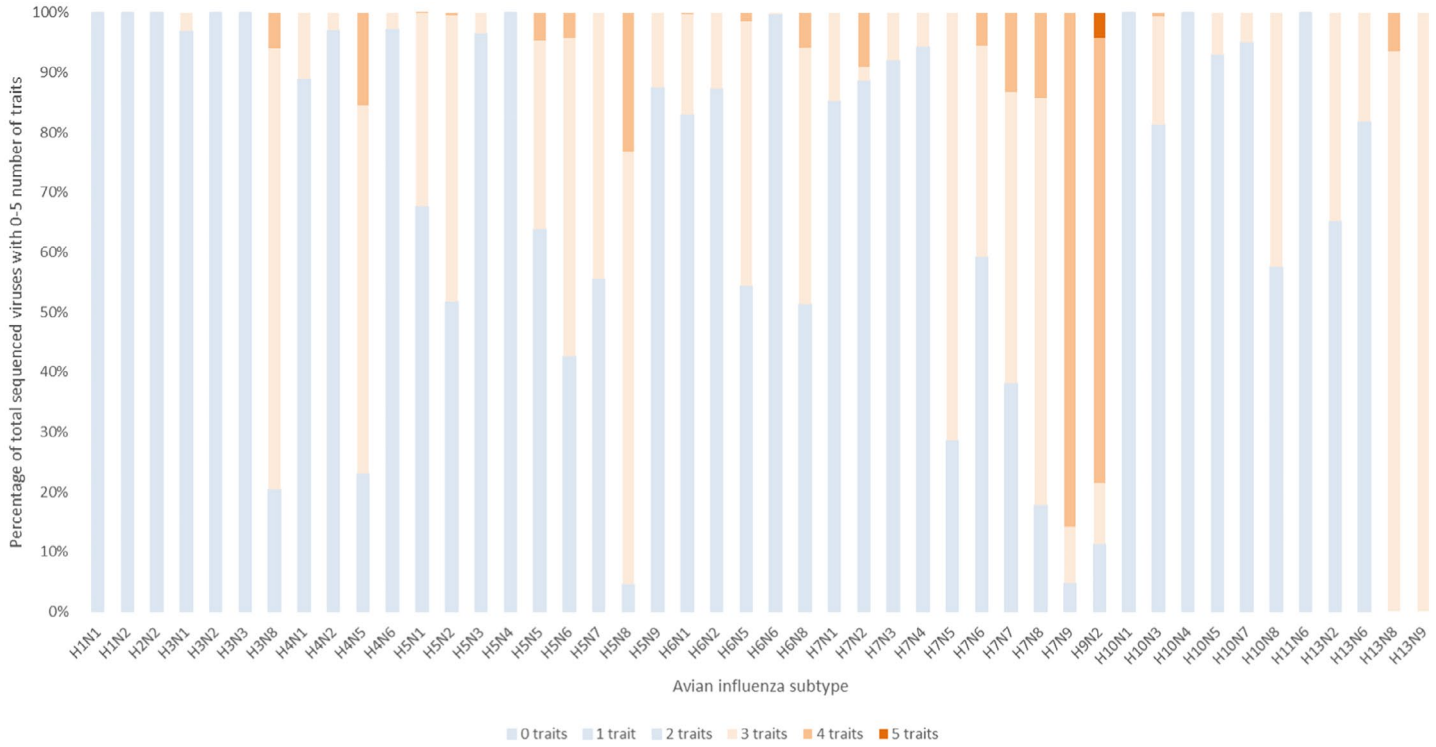

FIGURE 9 Proportion of trait counts (total number of 0–5 accumulated traits per virus) for the different influenza virus subtypes, displayed as a percentage of the total sequences for each subtype (0–2 traits are grouped into the same category, light blue).

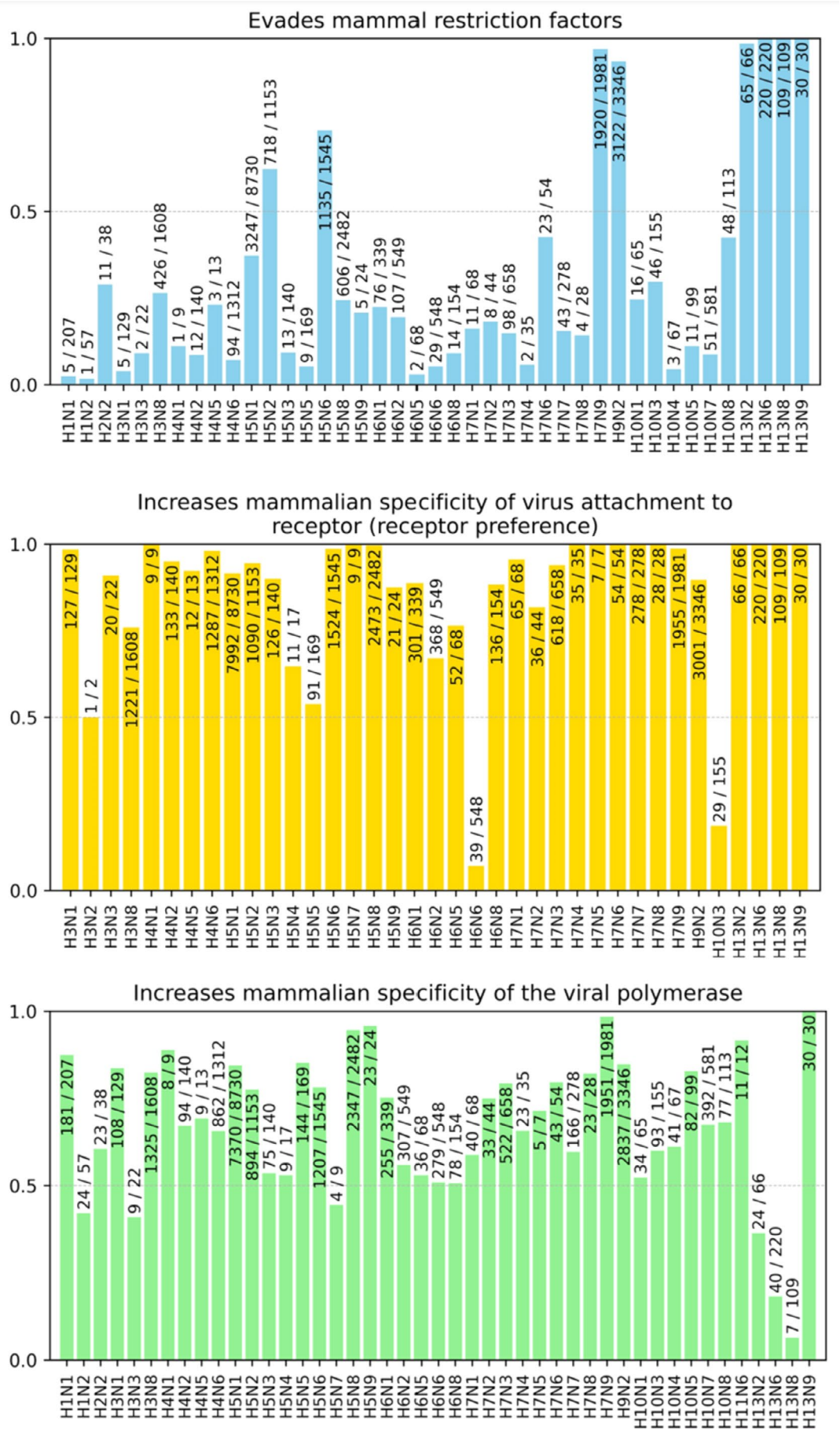

FIGURE 10 Proportion (0–1) of each trait across subtypes (34 mutations from subset 1). The total number of sequences containing at least one of the trait-associated mutations (n) on the total number of sequences (N) per subtype is displayed for each subtype and each trait (n/N) (the first three traits are shown; the other two are shown in Figure 11 for reason of space).

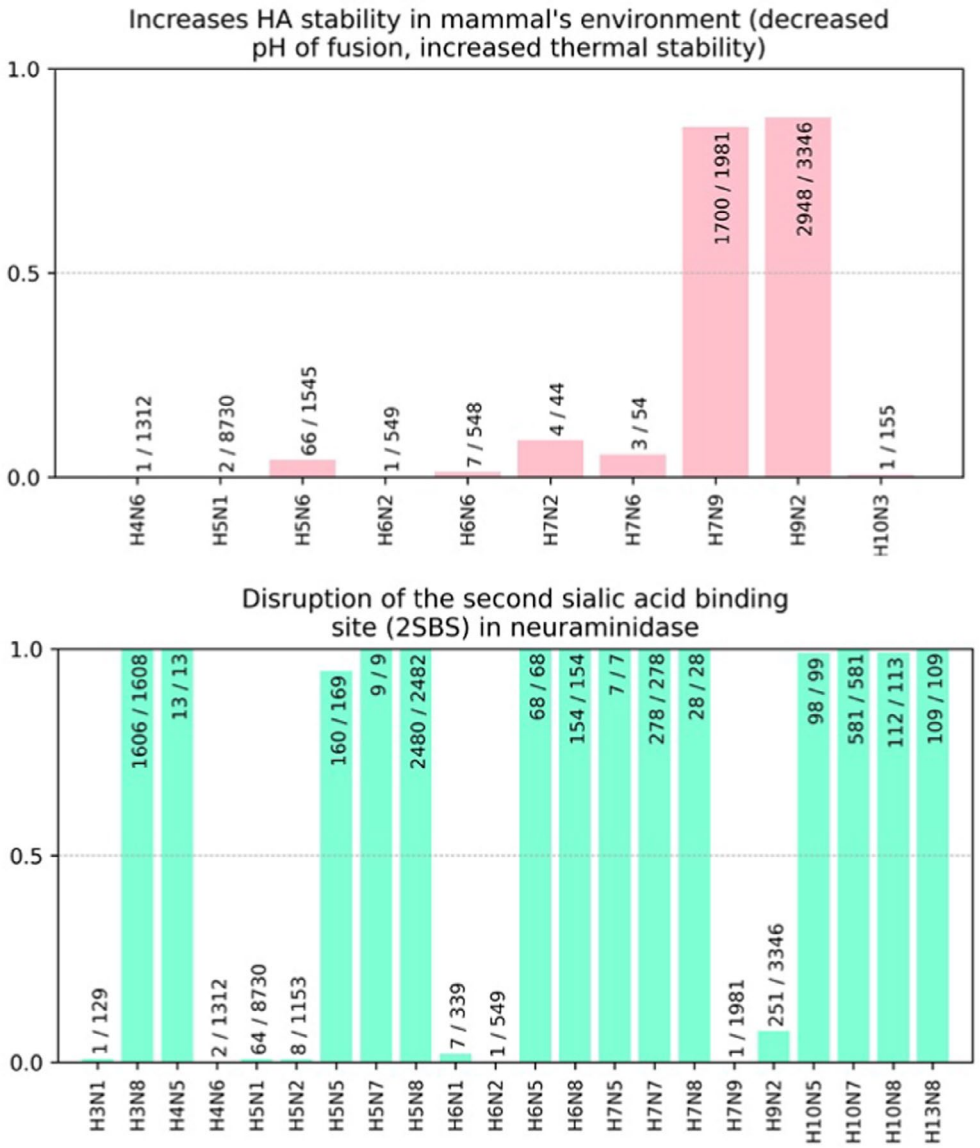

FIGURE 11 Proportion (0–1) of each trait across subtypes (34 mutations from subset 1). The total number of sequences containing at least one of the trait-associated mutations (n) on the total number of sequences (N) per subtype is displayed for each subtype and each trait (n/N).

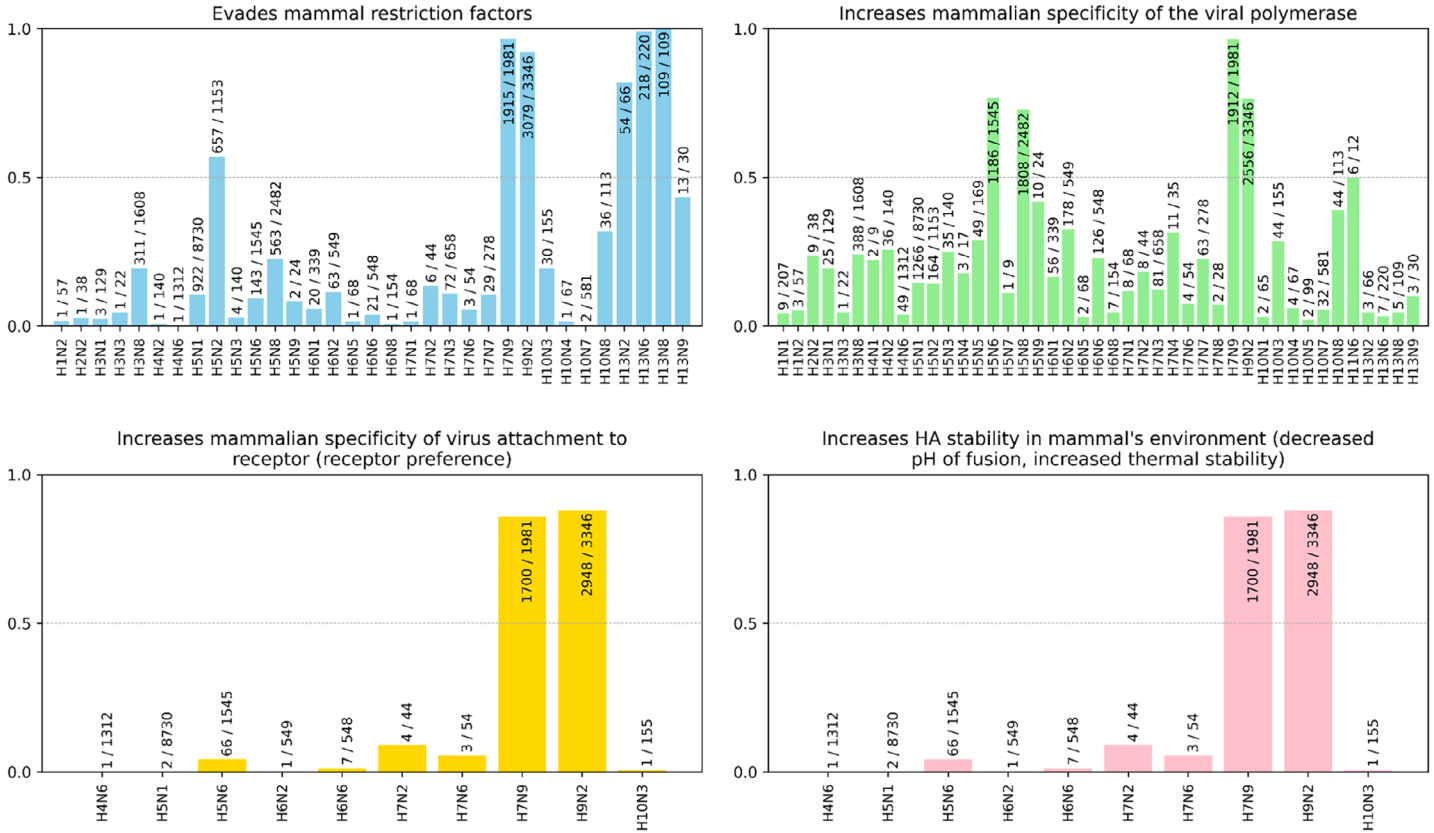

FIGURE 12 Proportion of each trait across subtypes (13 mutations, subset 2). The total number of sequences containing at least one of the trait-associated mutations (n) on the total number of sequences (N) per subtype is displayed for each subtype and each trait (n/N).

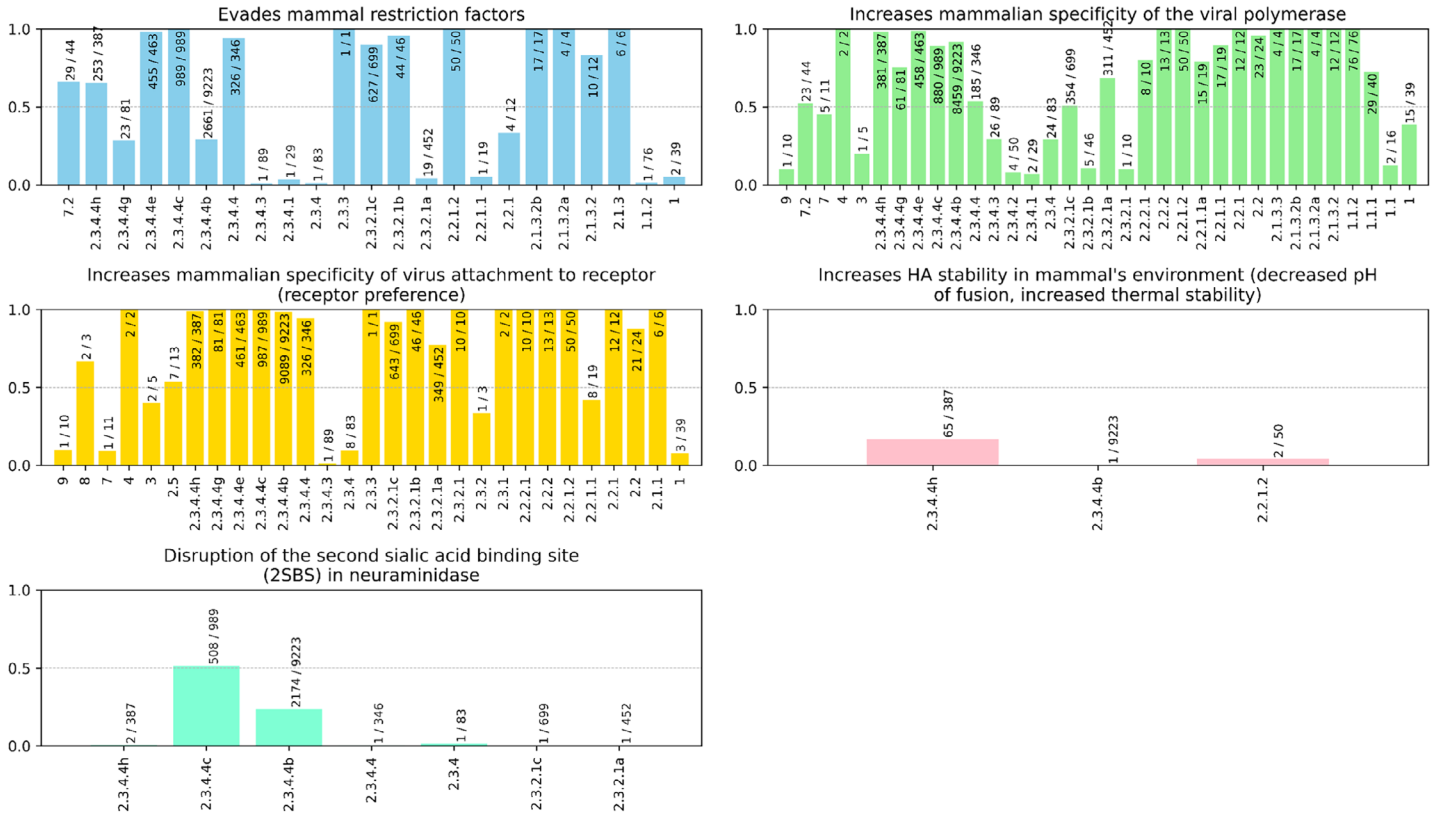

FIGURE 13 Proportion of each trait across H5 clades (34 mutations from subset 1). The total number of sequences containing at least one of the trait-associated mutations (n) on the total number of sequences (N) per subtype is displayed for each subtype and each trait (n/N).

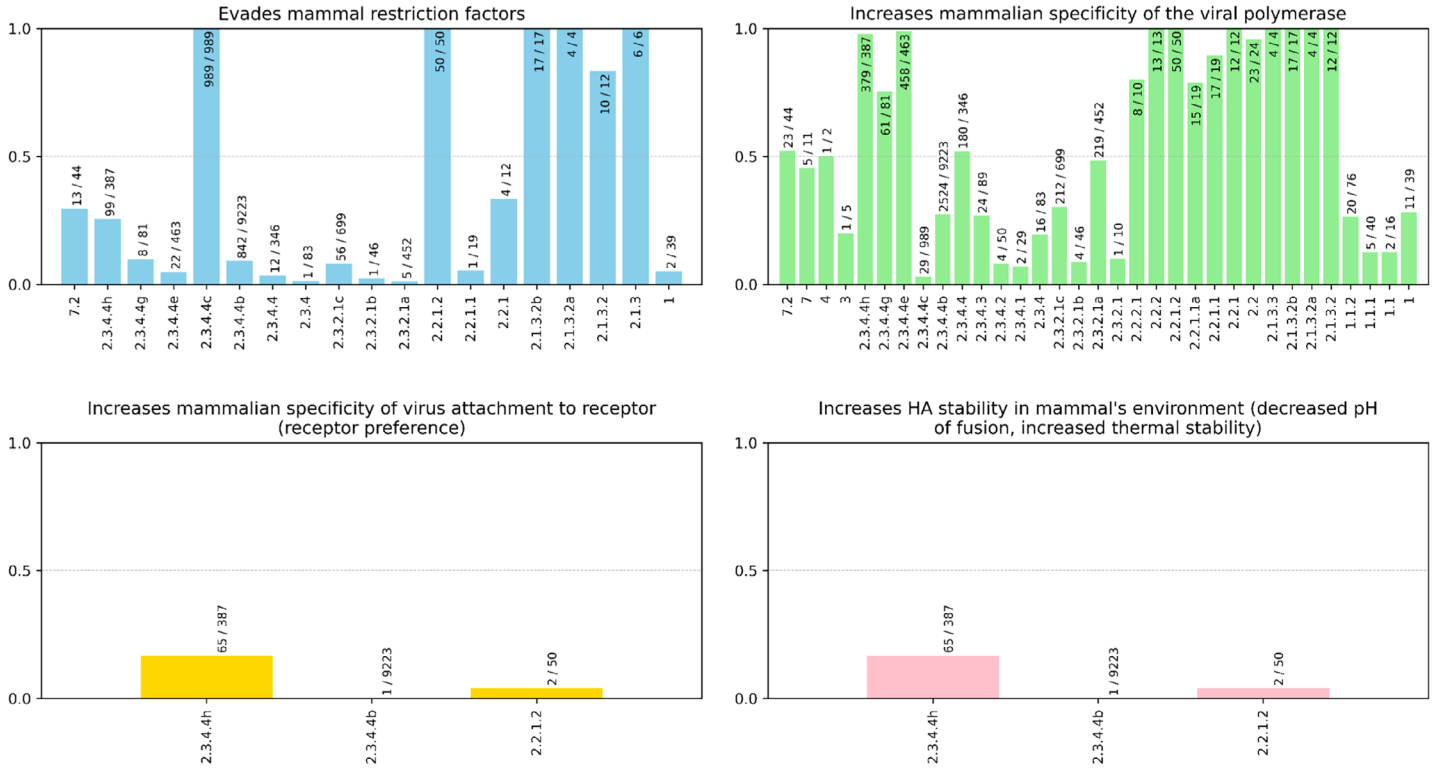

FIGURE 14 Proportion of each trait across H5 clades (13 mutations, subset 2). The total number of sequences containing at least one of the trait-associated mutations (n) on the total number of sequences (N) per subtype is displayed for each subtype and each trait (n/N).

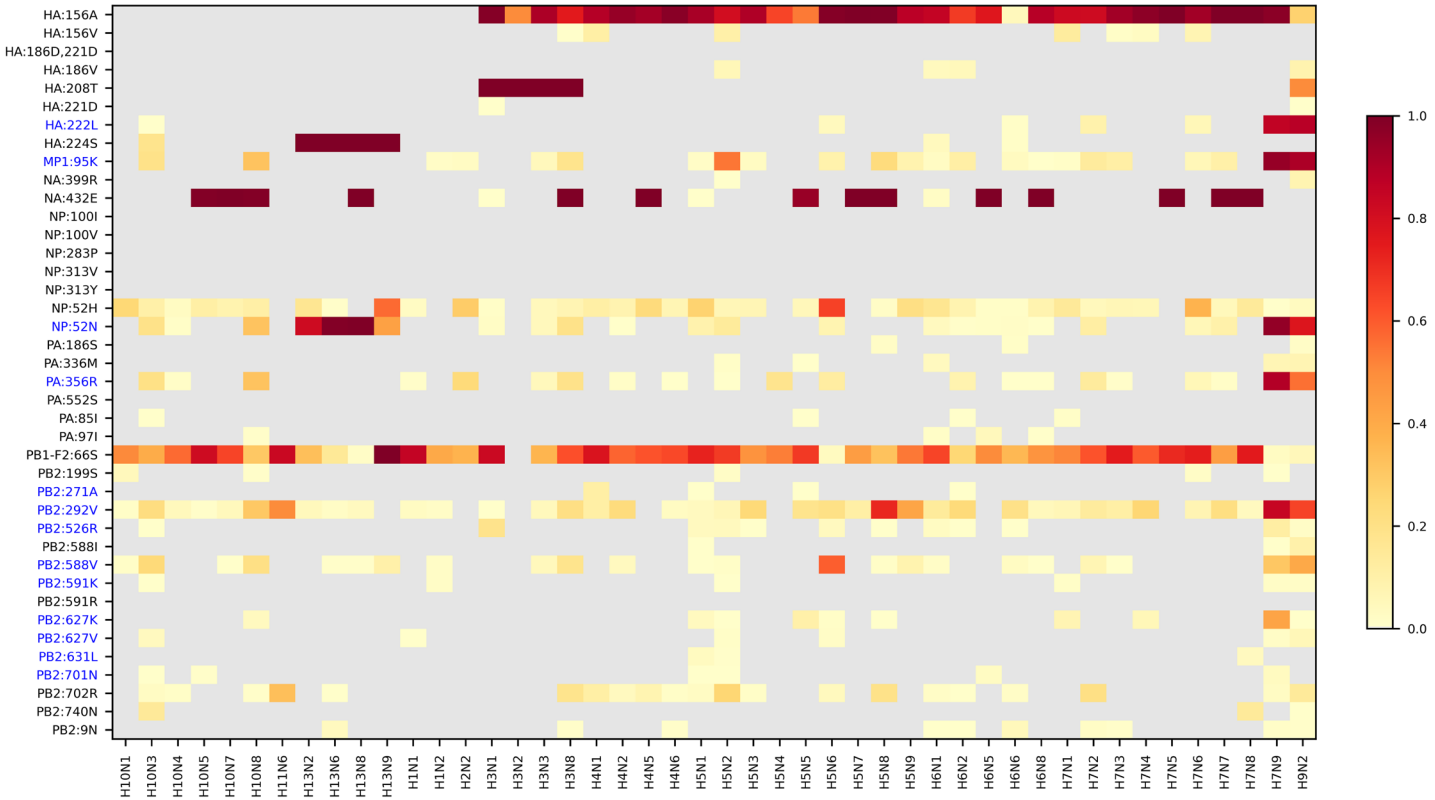

FIGURE 15 Heat map of the proportion of the 40 shortlisted mutations (subset 1) across subtypes (the 13 mutations, subset 2, are indicated in blue).

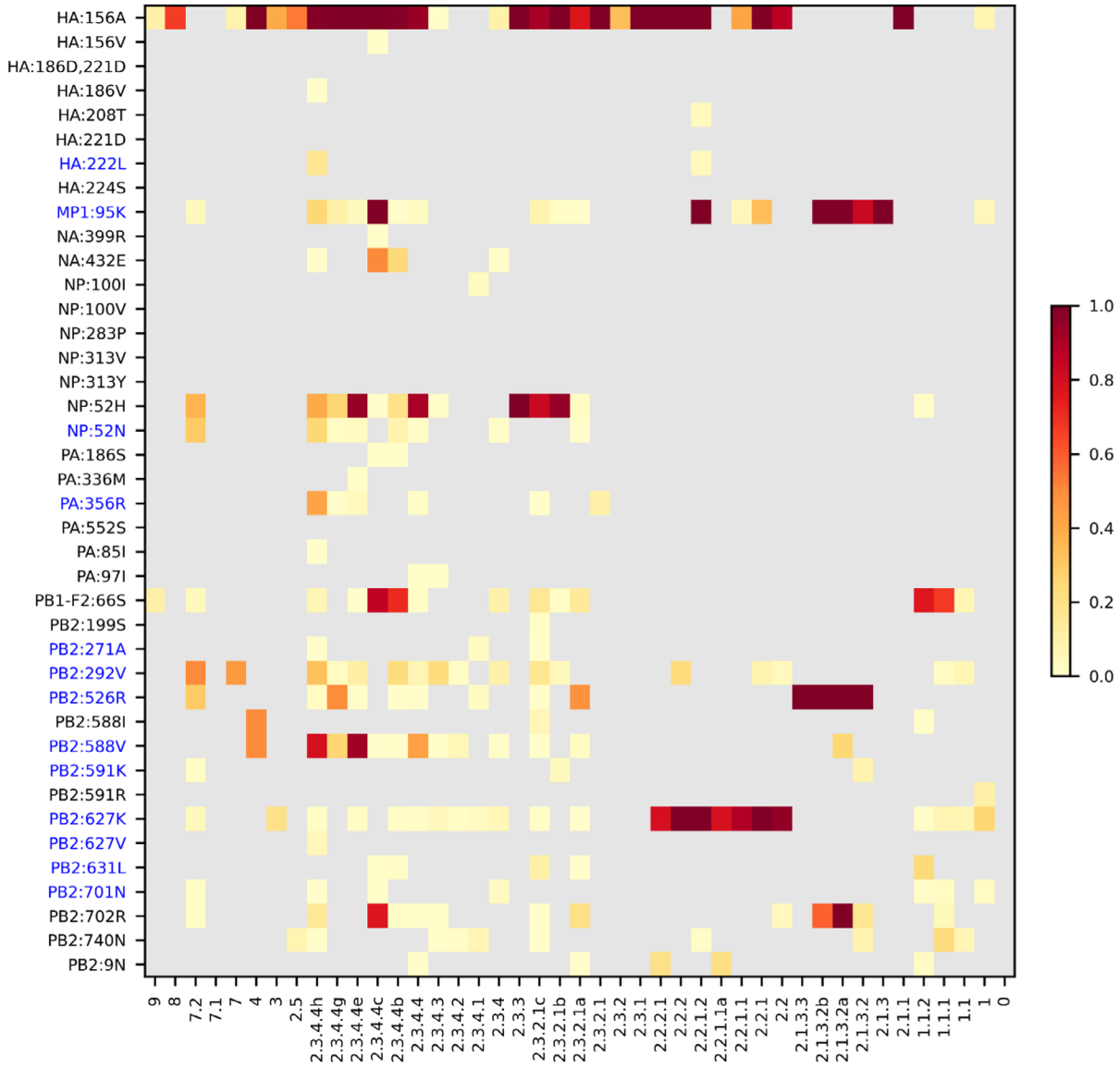

FIGURE 16 Heat map of the proportion of the 40 shortlisted mutations (subset 1) across H5 clades (the 13 mutations, subset 2, are indicated in blue).

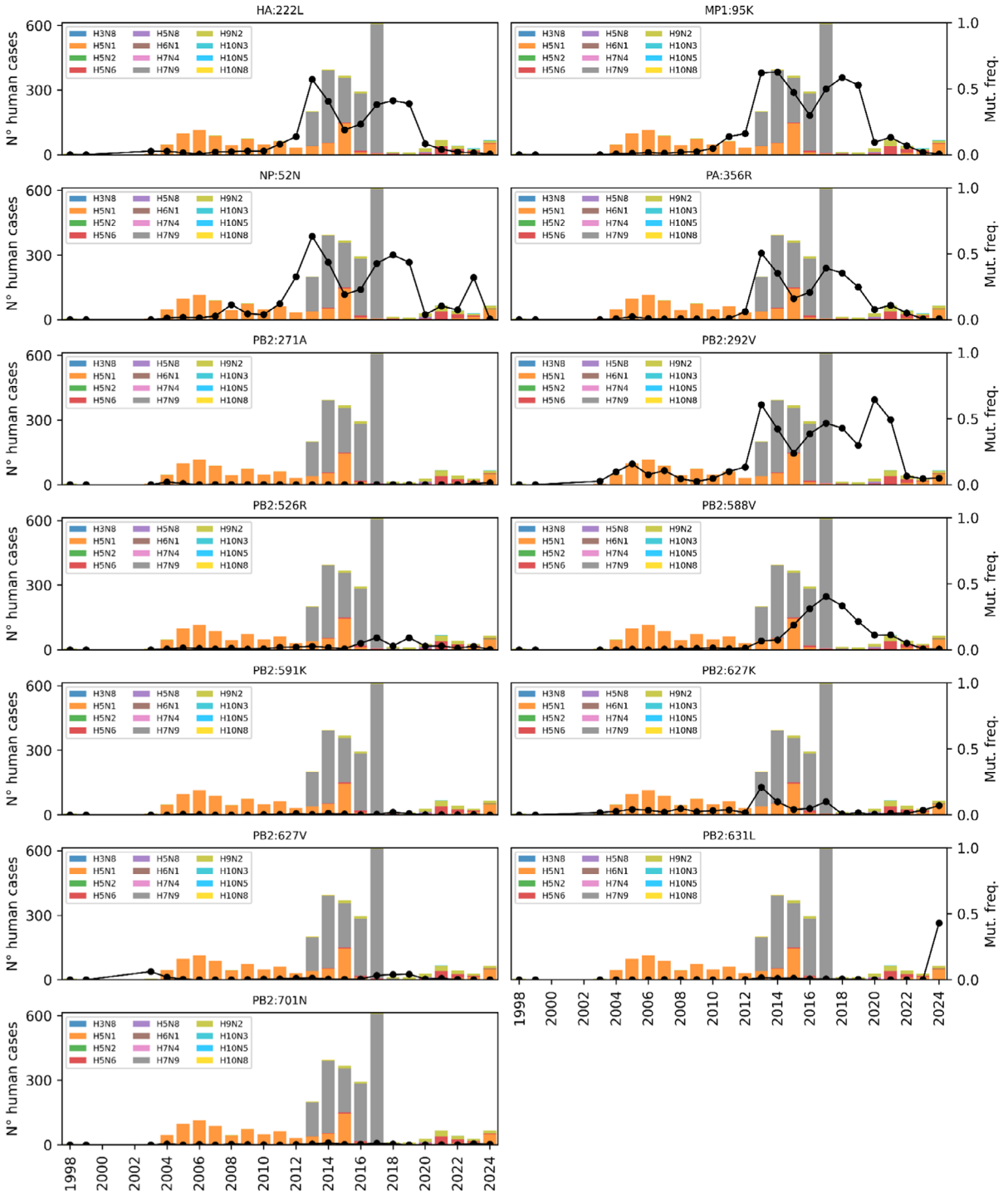

FIGURE 17 Timeline of the frequency of the 13 mutations (subset 2) in all the analysed viruses plotted over the number of human cases. Each plot represents the timeline (year) of the relative frequency (black line and right *y*-axis) of each of the 13 shortlisted mutations (indicated above each graph). The bars represent the number of AIV human cases reported each year, coloured according to the subtype (left y-axis).

4.5 | Summary of the WHO's TIPRA assessment

A systematic risk assessment approach is essential for evaluating the relative risk of influenza A viruses (IAVs) with pandemic potential. To achieve this, the TIPRA was developed under the Global Influenza Programme (GIP) of the WHO. GIP developed the TIPRA, based on the Influenza Risk Assessment Tool (IRAT) developed by the WHO Collaborating Centre for Surveillance, Epidemiology and Control of Influenza at the United States Centers for Disease Control and Prevention (CDC) (Burke & Trock, 2018; CDC, online-b). Ten risk items were used to characterise the overall risk: four related to virus properties (genomic characteristics, receptor-binding properties, transmissibility in animals and susceptibility to antivirals), four related to human population attributes (human infection, severity, population immunity (likelihood and impact)) and two related to virus ecology and epidemiology (geographical distribution in animals and infection in animals). Since its release, TIPRA has been used to assess the pandemic risk (likelihood and impact) of zoonotic IAVs of subtypes A(H5N6) clade 2.3.4.4,

A(H5Nx) clade 2.3.4.4, A(H5N1) clade 2.3.2.1c, A(H9N2) lineages Y280 and G1, A(H7N9), A(H1N1), A(H1Nx) lineage 1C (Yamaji et al., 2024).

A(H7N9) and A(H9N2) were found to have the highest overall likelihood scores, while A(H5N1), A(H5N6) and A(H7N9) were found to have the highest overall impact scores because of a high mortality among recorded cases (Yamaji et al., 2024).

A(H7N9) viruses had the highest relative risk at the time of assessment, highlighting the importance of continuous monitoring and reassessment as changes in epidemiological trends within animal and human populations can alter risk profiles.

For A(H9N2) viruses, experts agreed on lower impact scores due to the generally mild disease course in humans and the lack of human infection clusters. There were, however, varying risks that affected the likelihood scores across specific lineages, reflecting the preference of some viruses to bind to human α2,6-linked sialic acid, whereas others retained the ability to bind to avian-type receptors and the differences in transmissibility in ferrets via respiratory droplets. Considering the direct and indirect pandemic risks associated with A(H9N2) viruses, changes in their biological properties might trigger further risk assessments.

In the assessment of clade 2.3.4.4b A(H5N1) viruses in 2021, given that all detected human cases were asymptomatic at the time of the assessment, the overall impact score was lower than for all other A(H5) viruses that have been scored. The increased geographical spread of this virus clade in avian populations and low population immunity resulted in high scores for respective risk items; however, these scores were partly offset by the lack of virus adaptation to human or mammalian hosts. The overall likelihood score was similar to the 2020 A(H5N1) score. Genotype B3.13 clade 2.3.4.4b virus assessments that caused human cases in the USA during cattle AI outbreaks are ongoing.

Notably, all six AIVs that were assessed using both TIPRA and IRAT tools generated similar results.

IRAT has published a new IRAT assessment for the AI A(H5N1) clade 2.3.4.4b genotype B3.13 virus for A/Texas/37/2024. Assessment resulted in a similar (moderate) risk compared with the previously assessed H5N1 clade 2.3.4.4b viruses (CDC, online-d).

It needs to be noted that TIPRA or IRAT are not tools for predicting the subtypes of zoonotic IAV that will cause the next pandemic. The overall risk scores represent risk evaluation based on data available during the process, which could change with the availability of new evidence-based data.

4.6 | Surveillance to address zoonotic risks of AI

Due to the intrinsic characteristic of AIVs to constantly mutate, adapt, reassort and evolve leading to changes in virulence and host spectrum, surveillance targeting humans and a broad range of animals (birds and mammals) hosts should be in place, not only to promptly detect the infection in different species but also to genetically and phenotypically characterise virus strains, monitor mutations and prevent epidemics in animals and humans. Surveillance in mammals should be targeted to detect strains that have the potential to be, or have already become, established within a mammalian host population. In addition, it is important to monitor mutations of public health importance, i.e. those conferring changes in antigenicity compared with the vaccine viruses and CVVs or antiviral drug resistance. The evolving field of environmental and wastewater surveillance applied to detect potentially zoonotic AI strains is also discussed in this chapter.

4.6.1 | Legislative framework for AI surveillance in species different from birds (not listed species) and notification

The Commission Delegated Regulation (EU) 2020/689,[13] which sets rules for surveillance, eradication programmes and disease-free status for certain listed and emerging diseases, in Annex II about surveillance of AI linked with Article 4(3), at section 10, it specifies that HPAI surveillance must include surveillance activities in kept and wild animals of species not listed (as opposed to listed species belonging to the Aves class[14]) when the epidemiological situation indicates that those species may constitute a risk for animal and human health.

The EU Animal Health Law (Regulation (EU) 2016/429) provides a legal basis for controlling and reporting animal diseases with zoonotic potential. According to that, HPAI in mammals may be classified as an emerging disease (Article 6), outbreaks should be immediately reported to European Commission and MSs, and emergency measures, surveillance and traceability should be applied (can be as strict as for poultry, including culling animals in affected farms) (Articles 257 and 258).

The EU requires MSs to report outbreaks of AIVs in animals in accordance with EU animal health regulations within 24 h. According to the Regulation (EU) 2018/1882, both HPAI and infection with LPAI are listed diseases. Similar to the requirements by the WOAH, the notification should focus on subtypes that pose significant risks to poultry, wildlife and, potentially, human health. The definition of disease agent has been defined in Annex I to Regulation (EU) 2020/689:

- HPAI: an IAV of H5 and H7 subtypes or any IAV with an intravenous pathogenicity index (IVPI) greater than 1.2 or an IAV

[13] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02020R0689-20231011&qid=1717056414920.

[14] Annex of Implementing regulation - 2018/1882 - EN - EUR-Lex.

of H5 and H7 subtypes with a sequence of multiple basic amino acids present at the cleavage site of the haemagglutinin molecule (HA0) that is similar to that observed for other HPAI isolates.
- LPAI: Any IAV of H5 and H7 subtypes that are not HPAIVs as subtypes potentially at risk for mutating into HPAI. These subtypes are notifiable to identify clusters of LPAI infections in poultry and to contribute to increasing knowledge on LPAIV posing a potential zoonotic risk. Other LPAIVs can be notified on a voluntary base.

In case of zoonotic infections of AI identified in humans before a known outbreak in animals, according to the EU legislative framework, a coordinated One Health approach between animal health, public health and food safety authorities should be followed (WHO, 2024). Key pieces of EU legislation are:

- Zoonoses Directive (Directive 2003/99/EC), which sets up a framework for the monitoring and reporting of zoonotic agents across the EU. It requires MSs to collect and share data on zoonotic diseases, like AI, from both human and animal populations.
- Decision on early warning and response (Decision No 1082/2013/EU, repealed by Regulation (EU) 2022/2371 on serious cross-border threats to health[15]), which mandates MSs to set up systems for early warning and response for serious cross-border health threats, which include zoonotic diseases like AI. If a zoonotic LPAI or HPAI strain is identified in humans, MSs must assess the risk and notify the ECDC and EFSA.
- All laboratory-confirmed AI cases in humans need to be reported according to EU regulations and they are notifiable to the early warning reporting system (EWRS); they need to also be reported to WHO under the International Health Regulations (IHR). Clusters of cases of severe acute respiratory disease of unknown or novel cause shall also be assessed (utilising Annex 2) and are notifiable. Human cases in the EU/EEA should also be reported through Epipulse and the European Surveillance System (TESSy).

4.6.2 | Criteria to trigger surveillance for AI in non-human mammals

Table 5 outlines the criteria and provides guidance for AI surveillance in different non-human mammal species for detecting (potentially) zoonotic strains. Each category specifies the conditions under which sampling and sequencing should be triggered, the types of animals targeted and the recommended sampling indications and metadata collection. Note that not all situations might be covered, but practical examples are given that may be extrapolated to other circumstances.

The columns in the table indicate scenarios in which sampling and sequencing should be carried out based on supporting evidence and/or high-risk circumstances (high-risk areas[16] or high-risk periods[17]), [18]with the +, ++ or +++ numbers indicating their ranking order in terms of probability of occurrence of the specific scenario, which, consequently, also drives where sampling and sequencing should be carried out more often.

[15]https://data.europa.eu/eli/reg/2022/2371/oj.

[16]Areas with a high density of poultry farms and/or with current or previous history of AI outbreaks.

[17]In Europe, the AI high-risk season is typically the winter months (e.g. October–April), when a higher peak of AI outbreaks is reported (EFSA, ECDC and EURL, 2024a).

[18]In the absence of dead or sick animals, sampling asymptomatic animals can be also considered based on decisions by local authorities.

TABLE 5 Sampling criteria and guidance for the surveillance of AI in non-human mammals.

Criteria to trigger surveillance of AI		Mammals found dead, sick or asymptomatic, with epidemiological connection with infected poultry (i.e. in contact with infected poultry, mixed farming with poultry, ingestion of infected poultry products, carcasses, etc.)	Mammals found dead, sick or asymptomatic with epidemiological connection with infected wild birds (i.e. in contact with dead wild birds that have been confirmed as cases of AI)	Mammals found dead, sick or asymptomatic with epidemiological connection with infected mammals or mammal products (such as carcasses, milk, etc.) (i.e. in contact with mammals or mammal products that have been confirmed as cases of AI)	Mammals found dead in high-risk areas and during high-risk periods of AI circulation (i.e. no clear epidemiological connection has been identified with infected poultry/wild birds/ mammals but outbreaks have been reported in poultry and wild birds in the area/period where/ when those mammals have been found)	Mammals showing unexplained clinical signs in high-risk areas and during high-risk periods of AI circulation (i.e. no clear epidemiological connection has been identified with infected poultry/wild birds/ mammals after investigation but outbreaks have been reported in poultry and wild birds in the area/period where/when those mammals have been found)
Animal groups to be sampled	Targeted/at-risk species (see Figure D.1)					
Wild mammals (carnivores and scavengers)	Free-ranging foxes, mustelids, seals, etc.	+	+	+	+++ e.g. wild mammals found dead in an area where infected wild birds/ poultry/mammals have been recently reported. e.g. wild mammals found dead in high-risk periods/areas and already screened for specific diseases as part of a surveillance campaign (such as rabies).	++ e.g. wild mammals retrieved at rescue centres in high-risk periods/areas, and showing unexplained neurologic and respiratory signs.
	Sampling and sequencing indications	If a few individuals are found dead or sick (such as in cases observed in foxes), all of them should be sampled and whole genome sequencing should be performed on all positive ones (that are suitable for sequencing) to allow the detection of the molecular features of the predominant variant. If a massive collection of individuals is found dead or sick (such as in cases observed in marine mammals), it can be assumed that outbreaks (defined by being close in time and space) are more likely to represent a single variant circulation than multiple incursions of different variants, although this is less certain due to the lack of comprehensive exposure history. Therefore, at least 10–15 individuals (one sample per individual) should be sampled per outbreak (found close in time and space) and whole genome sequencing should be performed on all positive ones (that are suitable for sequencing) to allow the detection of the molecular features of the predominant variant. More samples can be taken according to laboratory capacity and pooled when testing and sequencing.				

TABLE 5 (Continued)

Criteria to trigger surveillance of AI		Mammals found dead, sick or asymptomatic, with epidemiological connection with infected poultry (i.e. in contact with infected poultry, mixed farming with poultry, ingestion of infected poultry products, carcasses, etc.)	Mammals found dead, sick or asymptomatic with epidemiological connection with infected wild birds (i.e. in contact with dead wild birds that have been confirmed as cases of AI)	Mammals found dead, sick or asymptomatic with epidemiological connection with infected mammals or mammal products (such as carcasses, milk, etc.) (i.e. in contact with mammals or mammal products that have been confirmed as cases of AI)	Mammals found dead in high-risk areas and during high-risk periods of AI circulation (i.e. no clear epidemiological connection has been identified with infected poultry/wild birds/ mammals but outbreaks have been reported in poultry and wild birds in the area/period where/ when those mammals have been found)	Mammals showing unexplained clinical signs in high-risk areas and during high-risk periods of AI circulation (i.e. no clear epidemiological connection has been identified with infected poultry/wild birds/ mammals after investigation but outbreaks have been reported in poultry and wild birds in the area/period where/when those mammals have been found)
Micromammals and bats	Rodents, bats, etc.	+++ e.g. rodents captured or found dead in an infected poultry farm, having potential contact with poultry carcasses in infected poultry farm	+	+++ e.g. rodents captured or found dead in an infected mammal farm	+	++ e.g. rodents showing unexplained mortality in high-risk periods/areas
	Sampling and sequencing indications	If a few individuals are found dead or sick, all of them should be sampled and whole genome sequencing should be performed on all positive ones (that are suitable for sequencing) to allow the detection of the molecular features of the predominant variant. Note that before sampling, it is important to verify that the mortality is not due to rodent control activities rather than HPAI. If a massive collection of individuals found dead or sick (this has not been observed so far), it can be assumed that outbreaks (defined by being close in time and space) are more likely to represent a single variant circulation than multiple incursions of different variants, although this is less certain due to the lack of comprehensive exposure history. Therefore, at least 10–15 individuals (one sample per individual) should be sampled per outbreak (found close in time and space) and whole genome sequencing should be performed on all positive ones (that are suitable for sequencing) to allow the detection of the molecular features of the predominant variant. More samples can be taken according to laboratory capacity and pooled when testing and sequencing. As above, first we need to ensure that the observed mortality is not related to rodent control activities.				
Companion animals	Cats, dogs, etc.	+++ e.g. companion animals in an infected poultry farm, fed with raw poultry meat in an infected poultry farm, having potential contact with poultry carcasses in infected poultry farm	++ e.g. dogs in contact with confirmed wild bird cases (e.g. positive dead wild ducks in forest, lands; positive found dead gulls on a beach, hunting dogs, etc.)	+++ e.g. companion animals in an infected cattle farm, fed with raw milk in infected cattle farm e.g. dogs in contact with confirmed wild mammal cases (e.g. seals found dead on a beach, etc.)	+	++ e.g. cats showing unexplained neurologic and respiratory signs in high-risk periods/ areas, e.g. cats showing unexplained neurologic and respiratory signs and other companion animals already been reported positive (e.g. Korean cats tested following the reports of infected cats in Poland)

TABLE 5 (Continued)

Criteria to trigger surveillance of AI		Mammals found dead, sick or asymptomatic, with epidemiological connection with infected poultry (i.e. in contact with infected poultry, mixed farming with poultry, ingestion of infected poultry products, carcasses, etc.)	Mammals found dead, sick or asymptomatic with epidemiological connection with infected wild birds (i.e. in contact with dead wild birds that have been confirmed as cases of AI)	Mammals found dead, sick or asymptomatic with epidemiological connection with infected mammals or mammal products (such as carcasses, milk, etc.) (i.e. in contact with mammals or mammal products that have been confirmed as cases of AI)	Mammals found dead in high-risk areas and during high-risk periods of AI circulation (i.e. no clear epidemiological connection has been identified with infected poultry/wild birds/ mammals but outbreaks have been reported in poultry and wild birds in the area/period where/ when those mammals have been found)	Mammals showing unexplained clinical signs in high-risk areas and during high-risk periods of AI circulation (i.e. no clear epidemiological connection has been identified with infected poultry/wild birds/ mammals after investigation but outbreaks have been reported in poultry and wild birds in the area/period where/when those mammals have been found)
	Sampling and sequencing indications	In case of companion animals, it is likely to be a small population so all individuals should be sampled and whole genome sequencing should be performed on all positive ones (that are suitable for sequencing) to allow the detection of the molecular features of the predominant variant.				
Domestic ruminants	Cattle, goats, etc.	+++ e.g. ruminants in infected poultry farm (mixed farming), fed with poultry litter	++ e.g. ruminants in contact with confirmed wild bird/animal cases found dead on the farm	++ e.g. ruminants with prior contact with infected ruminants or other domestic or wild animals or through movement of animals	+	++ e.g. ruminants showing unexplained signs (more common aetiology to be first excluded), e.g. decreased lactation, reduced feed intake, neurologic and respiratory signs, in high-risk periods/ areas
	Sampling and sequencing indications	In case of domestic ruminants (such as cattle, goats, etc.), multiple incursions of different variants within the farm at the same time is a rare event, making single variant circulation more likely than multiple variants. Therefore, approximately 10–15 individuals (one sample per individual) and bulk milk per epidemiological unit (herd) should be sampled and whole genome sequencing should be performed on all positive ones (that are suitable for sequencing) to allow the detection of the molecular features of the predominant variant. More samples can be taken according to laboratory capacity and pooled when testing and sequencing. Perform repeated sampling and sequencing over time of the same epidemiological units to monitor strain evolution. Note that in the context of an ongoing outbreak, pre-movement sampling and testing of domestic ruminants is required before they are moved to other farms. Whole genome sequencing should be performed on all positive ones (that are suitable for sequencing).				
Fur animals	Farmed mink, foxes, raccoon dogs, etc.	+	+++ e.g. fur animals in contact with confirmed wild bird cases found dead on the farm	++ e.g. fur animals with epidemiological connection with infected fur farms through movements of materials, people, etc.	++ e.g. fur animals found dead in high-risk periods/areas and already screened for specific diseases (e.g. SARS-CoV2) as part of specific surveillance campaigns	++ e.g. fur animals showing unexplained neurological and respiratory signs, especially in high-risk periods/areas

TABLE 5 (Continued)

Criteria to trigger surveillance of AI		Mammals found dead, sick or asymptomatic, with epidemiological connection with infected poultry (i.e. in contact with infected poultry, mixed farming with poultry, ingestion of infected poultry products, carcasses, etc.)	Mammals found dead, sick or asymptomatic with epidemiological connection with infected wild birds (i.e. in contact with dead wild birds that have been confirmed as cases of AI)	Mammals found dead, sick or asymptomatic with epidemiological connection with infected mammals or mammal products (such as carcasses, milk, etc.) (i.e. in contact with mammals or mammal products that have been confirmed as cases of AI)	Mammals found dead in high-risk areas and during high-risk periods of AI circulation (i.e. no clear epidemiological connection has been identified with infected poultry/wild birds/ mammals but outbreaks have been reported in poultry and wild birds in the area/period where/ when those mammals have been found)	Mammals showing unexplained clinical signs in high-risk areas and during high-risk periods of AI circulation (i.e. no clear epidemiological connection has been identified with infected poultry/wild birds/ mammals after investigation but outbreaks have been reported in poultry and wild birds in the area/period where/when those mammals have been found)
	Sampling and sequencing indications	In case of fur animals (such as minks, foxes, etc.), multiple incursions of different variants within the farm at the same time is a rare event, making single variant circulation more likely than multiple variants. Therefore, approximately 10–15 individuals (one sample per individual) per epidemiological unit (herd) should be sampled and whole genome sequencing should be performed on all positive ones (that are suitable for sequencing) to allow the detection of the molecular features of the predominant variant. More samples can be taken according to laboratory capacity and pooled when testing and sequencing. Perform repeated sampling and sequencing over time of the same epidemiological units to monitor strain evolution.				
'Other' domestic mammals	Rabbits, alpacas, horses, pigs, etc.	+++ e.g. domestic mammals in infected poultry farm (mixed farming), fed with poultry litter	++ e.g. mammals in contact with confirmed wild bird cases found dead on the farm	++ e.g. alpacas with epidemiological connection with other infected mammal farms through movements of materials, people, etc.	+	++ e.g. domestic mammals showing unexplained neurologic and respiratory signs in high-risk periods/ areas
	Sampling and sequencing indications	In case of other domestic animals (such as rabbits, alpacas, etc.), multiple incursions of different variants within the farm at the same time is a rare event, making single variant circulation more likely than multiple variants. Therefore, approximately 10–15 individuals (one sample per individual) per epidemiological unit (herd) are to be sampled and whole genome sequencing should be performed on all positive ones (that are suitable for sequencing) to allow the detection of the molecular features of the predominant variant. More samples can be taken according to laboratory capacity and pooled when testing and sequencing. Perform repeated sampling and sequencing over time of the same epidemiological units to monitor strain evolution.				
Metadata collection		Species, date and location of sampling, clinical information, exposure context				

4.6.3 | Surveillance in wild mammals

4.6.3.1 | *Wild carnivores and scavengers*

For wild mammals (Table 5), species that have been reported most frequently infected (mainly carnivores, see Section 4.1) should be especially targeted for sampling, such as foxes, mustelids (badgers, otters, mink, etc.), seals and other marine mammals (ENETWILD consortium et al., 2024a).

For wild mammals, surveillance criteria are the following:

- Surveillance should be triggered if wild mammals are **found dead** in **high-risk** areas and during **high-risk periods** of AI circulation, i.e. in an area where infected wild birds, poultry or mammals have been recently reported (e.g. up to 15 days, and with the timing defined according to the specific epidemiological situation). Examples include infected seals coinciding with infected wild coastal birds (Puryear et al., 2023) and massive mortality events of sea lions observed in Argentina, Peru and Chile (Plaza et al., 2024) during high-risk periods of AI circulation. Surveillance should also take place for wild mammals found dead in high-risk periods/areas (e.g. by hunters) and which are already screened for specific diseases (opportunistic surveillance) as part of surveillance campaigns (such as rabies).
- Surveillance should also be triggered for wild mammals showing **unexplained clinical signs** in **high-risk areas** and during **high-risk periods** of AI circulation. This includes wild mammals retrieved at rescue centres during high-risk periods/areas and showing unexplained neurologic and respiratory signs after investigations, which could be indicative of HPAI (ENETWILD consortium et al., 2024a).
- Surveillance is also necessary when wild animals are found near infected poultry farms/wild birds/mammals especially if found sick or dead, but also if found asymptomatic (e.g. if they are trapped). However, this is not very likely due to the limited probability of occurrence of this scenario and the difficulty in accessing comprehensive exposure history in wildlife. It may be possible for wild mammal populations that are closely monitored, such as those under research or conservation programmes.

The sampling approach is reported in Table 5.

4.6.3.2 | *Micromammals and bats*

For other small wild mammals, such as rodents and bats, limited information is available on their infection with currently circulating HPAI (clade 2.3.4.4b) viruses (Velkers et al., 2017).

Rodents (such as mice, rats and voles) are present in high numbers not only in rural, but also in urban and peri-urban areas and they serve as vectors and reservoirs of a large number of infectious organisms, which may cause diseases both in animals and humans. The existing scientific literature does not report sufficient data on the susceptibility of 'wild' free-living rodents to the infection. However, recent reports indicate that house mice (*Mus musculus*), collected from an infected poultry premise and orally inoculated with infected milk sample tested positive for HPAI, suggesting that rodents could play a role in the HPAI epidemiology. Bank voles (*Myodes glareolus*) have also been shown to be susceptible to HPAI A(H5)/A(H7) infection and to shed the virus via the nasal route without showing any evident signs/symptoms of the disease. As well, the viral excretion was sufficient to infect other voles which had entered into contact with the infected ones (Eisfeld et al., 2024; Guan et al., 2024; Romero Tejeda et al., 2015).

Bats have been identified as potential carriers of unique influenza viruses that exhibit several unique features that distinguish them from conventional IAVs, including A(H17N10) and A(H18N11) found in New World bats (Yang et al., 2021). In an experimental setting, A(H18N11) has shown poor replication in laboratory animals, such as mice and ferrets, but can infect certain bat species, causing mild symptoms. This apparent limited pathogenicity and host range may indicate adaptation to specific bat hosts rather than broader zoonotic potential. However, in 2017, a novel IAV was isolated from an Egyptian fruit bat and was related to avian A(H9N2) viruses, probably being the result of a bird-to-bat transmission event (El-Shesheny et al., 2024; Halwe et al., 2024). The discovery of influenza viruses in bats of avian origin, and their possession of some biologic properties typically associated with human influenza viruses, highlights the importance of surveillance for AIVs with zoonotic potential in bats to understand their epidemiology and potential impact on public health.

For other small wild mammals, surveillance criteria are the following:

- Surveillance should be triggered for any small wild mammals (primarily found dead, sick animals and, in the absence of those, sampling asymptomatic animals can be also considered based on the decision of local authorities) with epidemiological connections to infected poultry or mammals. For example, if rodents are captured (probably asymptomatic in this case) or found dead inside or in proximity to infected poultry or mammal farms. It is generally assumed that they are abundant inside (numbers are highly dependent on biosecurity measures) and around most farms and can be in contact with carcasses; therefore, exposure should be assumed to be likely and infection possible depending on the circulating virus characteristics.
- Surveillance should also be triggered for small wild mammals showing **unexplained clinical signs** in **high-risk areas** and during **high-risk periods** of AI circulation, such as rodents showing unexplained mortality in high-risk periods/areas. The sampling approach is reported in Table 5.

Further insight into HPAI surveillance indication for wild mammals are provided by ENETwild,[19] where it is recommended not to neglect other species other than carnivores (large carnivores, mesocarnivores and mustelids), such as marine mammals and wild boar.

4.6.3.3 | *Surveillance in wildlife facilities: Wildlife rescue centres and zoos*

Wildlife rescue centres usually recover a variety of species of sick and injured wild animals, rehabilitate them and subsequently release them back into the wild. During outbreaks of HPAI, cases have been documented in wild birds and mammals within rescue centres over recent years (Caliendo et al., 2022; Floyd et al., 2021; Hall et al., 2024). Rescuing wild animals during outbreaks of HPAI can be particularly risky due to the high infection threat posed by the animal collection and the risk of spillover events occurring in such facilities (Floyd et al., 2021). Moreover, human exposure to these animals presents a greater opportunity for a potential zoonotic virus to infect humans than that for the same virus in the wild. There are no established common guidelines for the surveillance and management of wild animals suspected of HPAIV infection.

Surveillance implemented at wildlife rescue centres could be complementary to other existing wildlife surveillance systems as they can detect positive sick or asymptomatic animals, unlike other systems that usually detect dead wild animals. Also, these centres tend to receive mostly urban to peri-urban animals; thus, this could lead to monitoring animals more at human–wildlife interface compared with wild populations monitored by other methods. However, while these centres might play a role in monitoring the health status of wildlife, existing studies on the role of rescue centres in HPAI surveillance are largely theoretical and not well integrated with current surveillance systems (Gourlay et al., 2014; Kelly et al., 2021; Randall et al., 2012).

Moreover, several aspects need to be considered if the objective is to implement surveillance in rescue centres. First, the network of rehabilitation centres varies between countries, resulting in a lack of standardisation in practices and resources. Many centres have limited financial resources, which poses a challenge if new surveillance activities are to be implemented. Their primary focus is on care rather than surveillance, requiring the raising of awareness, acceptability and training for this new activity.

However, according to the sick or injured species received by the rescue centres, surveillance could be conducted according to information provided in Table 5. In particular, regular checks of species frequently found infected in the wild or animals displaying clinical signs attributable to AI could be performed at wildlife rescue centres during high-risk periods and areas of AI circulation. Additionally, rescue centres seem particularly interesting for passive surveillance driven by pathognomonic clinical signs since they can potentially aggregate sick cases. In zoos, the level of risk for AI transmission and spread is lower than in rescue centres as animals undergo veterinary checks before being introduced or moved between zoos, and movements are generally limited. Biosecurity is also generally good because of animal value and their captivity is constantly monitored by zoo personnel. Thus clinical signs in animals may be easily spotted and testing can be performed.

4.6.3.4 | *Type of sample and diagnostic test for HPAI in wild animals*

HPAI A(H5Nx) viruses are more neuropathogenic than other IAVs in mammals, with some variability within the lineages of the Gs/GD (Bauer et al., 2023), being the severe neurological disease in mammals related to the neuroinvasive and neurotropic potential of HPAI A(H5Nx) viruses. The Gs/GD H5 lineage, first identified in 1996, marked a notable shift in the pathogenicity and neurotropic characteristics of AIVs as, before its emergence, neuroinvasion was not commonly associated with influenza viruses. This makes the central nervous system (CNS) a preferred site for sampling HPAI-suspected wild mammals. A high virus detection rate in CNS has been repeatedly observed in wild carnivores and has been associated with the route of infection that might involve ascending infection by the olfactory nerve (Bauer et al., 2023). Collection of CNS samples is also recommended in marine mammals as there is multiple evidence that viral replication mainly occurs in the brain with only little systemic virus dissemination, despite possible differences in the route of invasion of the CNS. This was observed mostly in toothed whales (odontocetes) (Murawski et al., 2024) that, with the exception of baleen whales (mysticetes), lack olfactory anatomy, thus eliminating the possibility of olfactory tract neuroinvasion by influenza virus. Therefore, a primary haematogenous route of entry into the CNS is suspected, with secondary neuron-to-neuron invasion. It is also of particular importance to note that, if CNS samples are collected under different surveillance programmes (e.g. rabies), caution should be used as not all brain parts might have the same amount of virus. For example, rabies testing usually focuses on the thalamus, pons and medulla as the most sensitive parts of the brain for rabies virus, while for AIV the olfactory bulb and frontal lobe usually have a higher concentration of viruses, although this is an inconsistent feature (Tammiranta et al., 2023). Other samples that should be collected for AIV testing in wild mammals include samples from the upper and lower respiratory tract, spleen and liver. Gastrointestinal tract samples or rectal swabs in both terrestrial and marine HPAI-affected mammals were found to have lower viral loads compared with the above-mentioned tissues. Although they may have lower viral loads, those methods (e.g. oral and cloacal swabs, environmental faecal samples, respiratory excretions from surface swabs) are considered non-intrusive and can be used to minimise stress and harm to the animals.

[19] https://zenodo.org/records/13885776.

Rodents are probably the mammals that will be captured in the largest numbers during most investigations, especially those living close to poultry facilities. Nonetheless, their typically small size will often limit the samples that can be collected from them. For example, conducting a nasal wash or nasal swab on a small rodent is generally not feasible due to their small size, but is typically the best sample that can be collected from a live mammal. Notably, titres from post-mortem nasal washes of experimentally infected house mice were typically orders of magnitude lower than selected tissues harvested from this species. Furthermore, oral swab samples were only rarely positive in this species and at very low levels if positive (Shriner et al., 2012). With these constraints in mind, selected tissues (e.g. lungs, nasal turbinates and trachea) may represent the best target sample when testing small rodents.

Based on the limited information available on AIV infection in bats, oropharyngeal and anal swabs should be collected as a minimum in bats, as transmission of New World bat-adapted AIVs appears to occur by the faecal–oral route rather than the respiratory route, which is common for other influenza viruses. The A(H9N2) viruses found in Old World Egyptian fruit bats instead were detected at similar rates from oral and anal swabs.

In live wild mammals, due to their elusive nature, especially carnivores, collection of samples can be logistically challenging. Moreover, the wide range of species with varying behaviours, habitats and ecological roles complicates the design of standardised monitoring protocols that can be effectively applied across different species and environments.

Screening for the M gene by real-time reverse transcription-polymerase chain reaction (RT-PCR) in wild mammals would allow the detection of AIVs with zoonotic potential. Further genetic analysis performed by WGS would allow the definition of genetic characteristics and the presence of zoonotic mutations.

Serological surveys could be useful to monitor the exposure to the virus of a certain animal population or species, although large numbers of samples might be difficult to collect in live wild mammals. Samples obtained from dead carcasses such as the cardiac blood clots, meat juices or lung juices might be used for serological analyses, although there is a lack of validation of these techniques for AIV on these samples. Serology, although useful to prove past exposure to AIV individuals or populations, does not make it possible to decipher the characteristics of the virus, and therefore, any serological studies should be interpreted as complementary to virological surveillance to increase confidence in surveillance sensitivity.

4.6.4 | Surveillance of companion animals in households or on the farm

For companion animals, those species that have been predominantly found infected in the past should be especially targeted for sampling, such as cats, dogs and ferrets (Domańska-Blicharz et al., 2023; Moreno et al., 2023; Sillman et al., 2023).

For companion animals, surveillance criteria are the following:

- Surveillance should be triggered for companion animals (found dead, sick or asymptomatic) with epidemiological connections to infected poultry or mammals. Examples are companion animals in contact with infected poultry or mammals in a farm fed with infected products, such as raw poultry meat in infected poultry farm or raw milk in an infected cattle farm. Also, this includes dogs in contact with confirmed cases of infected wild mammals such as seals. Priority should be given to sick or found dead companion animals, but surveillance should be also conducted on asymptomatic animals. Indeed, asymptomatic infection has been shown in cats and dogs, often sampled because of their presence on infected poultry farms (Moreno et al., 2023). Infection through the ingestion of raw poultry meat was demonstrated in indoor cats in Poland (Rabalski et al., 2023). However, in other cases, cats were found positive without documented exposure to food with raw poultry meat or contact with the outside environment (Domańska-Blicharz et al., 2023). On several infected dairy farms in the USA, HPAI cases were reported in cats and, in some cases, affected animals showed neurological signs. Consumption of colostrum or raw milk was identified as a likely source of infection (Hu et al., 2024).
- Surveillance should be triggered for companion animals (found dead, sick or asymptomatic) with epidemiological connection to infected wild birds. Examples are companion animals in contact with confirmed wild bird cases, such as (hunting) dogs in contact with positive dead wild ducks in wetlands, ponds or parks or with dead gulls found on a beach. Priority should be given to sick or found dead companion animals, but surveillance should be also conducted on asymptomatic ones.
- Surveillance should also be triggered for companion animals showing unexplained clinical signs (in particular if several animals are affected) in high-risk areas in the country with reported infection in poultry or mass mortality in wild birds and during high-risk periods of AI circulation. In particular, this includes companion animals showing unexplained neurologic and respiratory signs during high-risk periods/areas after investigations, as was the case for most infected cats (Domańska-Blicharz et al., 2023), in particular during high-risk periods of AI circulation. Other signs have been reported: serosanguinous nasal discharge and icterus, convulsions, ataxia or other neurological signs, as well as gastrointestinal symptoms (Briand et al., 2023; Domańska-Blicharz et al., 2023).

The sampling approach is reported in Table 5.

4.6.4.1 | *Type of sample and diagnostic test for HPAI in companion animals*

Until 2004, when outbreaks occurred in Thailand with several fatalities among tigers, domestic cats and other felids, little attention was given to felines, as previous experiments had shown that cats were not important hosts for AI and, although they could be infected by human viruses or AIVs, they rarely showed clinical signs (Hinshaw et al., 1981; Kuiken et al., 2006). Since then, numerous naturally occurring or experimental infections with HPAI and LPAI have been reported in domestic and captive felids (Sun et al., 2015). If clinical signs are present, samples from the respiratory tract should be privileged, as viral replication in the respiratory system has been reported for different subtypes of AI. From fatal cases, sampling should include the lower respiratory tract as well as nervous tissues, being the tissues more frequently affected in zoonotic cases (Klopfleisch et al., 2007; EFSA, ECDC and EURL, 2023; Driskell et al., 2013; Frymus et al., 2021), together with any tissue in which pathological changes have been observed. Serological surveys could be implemented to evaluate the introduction of zoonotic AIVs in cat shelters using appropriate techniques such as HI and serum neutralisation assays, which can differentiate immunological responses at the subtype level. Compared with felids, dogs appear to be more resistant to infection, which occurs mostly asymptomatically as suggested by experimental infections and serological evidence. In the few reports available of experimental infection by zoonotic AIV detection of viral replication in nasal swabs has been successful only shortly after infection at very low titres (Lyoo et al., 2017; Yuk et al., 2017). In a report of a fatal HPAI A(H5N1) infection in a dog, the virus was isolated from lung, liver, kidney and urine specimens, but not from ground brain tissue (Songserm et al., 2006).

The selection of tests for the detection of zoonotic AIVs should consider that dogs might harbour canine influenza viruses for which they represent the maintenance hosts. The canine influenza viruses belong to the A(H3) HA subtype (i.e. A(H3N2) and A(H3N8)) and, therefore, in case of detection of an H3 virus, a complete genome characterisation is warranted to fully understand the genomic composition of the virus. Other subtypes do not routinely circulate in this species, and therefore, any positive result should be considered as a spillover infection. Similarly, the detection of antibodies against type A influenza should be further characterised at the subtype level due to the possible presence of antibodies related to exposure to field strains of canine influenza viruses or to vaccination against canine flu.

Cats and felids instead are not considered maintenance hosts of any AIV subtype and therefore, for surveillance purposes, generic AI tests can be used for the detection of infection by AIVs. Any positive sample in generic AI tests should be then sequenced by WGS for the definition of genetic characteristics of the infecting virus and for the detection of zoonotic mutations.

4.6.5 | Surveillance in domestic ruminants

Domestic ruminants that have been predominantly found infected by AI are cattle, as occurred in the outbreaks reported in the USA in 2024 (Section 4.1). Due to the epidemiological characteristics of the AI spread in this species and setting, with the involvement of milk, milking equipment, fomites, etc., dairy cattle should be targeted by surveillance under the criteria explained below. For domestic ruminants, surveillance criteria are the following:

- Surveillance should be triggered for domestic ruminants **primarily found dead and sick animals** (and in the absence of dead or sick animals, sampling asymptomatic animals can be also considered based on decisions by local authorities) with **epidemiological connections** to **infected poultry**, such as domestic ruminants in contact with infected poultry (mixed farming environments). For example, reported infected goats in the USA were in close contact with infected poultry.[20]

The same surveillance strategy would apply to:

- Domestic ruminants with **epidemiological connections** to **infected wild birds**. Examples are ruminants in contact with confirmed wild bird cases found dead on the farm.
- Domestic ruminants with **epidemiological connections** to **infected mammals**, such as domestic ruminants have prior contact with infected ruminants through the movement of animals.
- Surveillance should be also triggered for domestic ruminants showing **unexplained clinical signs** (for example mastitis) in **high-risk areas such as regions with reported infection in poultry** and during **high-risk periods** of AI circulation, such as domestic ruminants showing unexplained decreased lactation, reduced feed intake, neurologic and respiratory signs during high-risk periods/areas (Ly, 2024; Sah et al., 2024) or in cases of unexplained unusual or mass mortality events, such as the unusual deaths in newborn goats in the USA.[21]

The sampling approach is reported in Table 5.

Further recommendations about surveillance of AI in dairy cattle are provided by FAO (El Masry et al., 2024), e.g. to maintain in all countries passive surveillance and to consider routine and opportunistic sampling to evaluate the health of cattle

[20] https://www.cidrap.umn.edu/avian-influenza-bird-flu/avian-flu-detected-first-time-us-livestock.
[21] https://www.cidrap.umn.edu/avian-influenza-bird-flu/avian-flu-detected-first-time-us-livestock.

populations, while, for at-risk countries, targeted or risk-based surveillance to assess cattle health if exposed to poultry or wild birds, and to investigate suspected outbreaks in cattle.

4.6.5.1 | *Type of sample and diagnostic test*

There is limited scientific literature on infection in ruminants, being traditionally considered resistant to infection from AIVs (Sreenivasan et al., 2019). In March 2024, the first HPAI A(H5N1) infection was observed in ruminants on a recently depopulated poultry farm in Minnesota, USA, where goats were in close contact with infected poultry and shared water sources and pasture. Five goat kids tested positive for HPAI A(H5N1) in multiple tissue samples, including the brain. Two days after the report on goats, cattle in two dairy farms, one in Kansas and one in Texas, tested positive for HPAI A(H5N1) (clade 2.3.4.4b). Until October 2024, 192 dairy farms had tested positive for HPAI A(H5N1) (Rodriguez et al., 2024) with further transmission between herds associated mainly with cattle movement. High viral loads have been found in milk, while moderate to low titres have been found in samples from the respiratory and gastrointestinal (GI) tracts. APHIS collected multiple tissues from the culled animals and, in 1/96 culled dairy cows,[22] the muscles tested positive. According to APHIS,[23] in total 109 muscle samples were collected and tested in May 2024. The samples were analysed by APHIS using PCR to determine the presence of viral particles. No viral particles were detected in 108 out of 109 muscle samples. Considering the limited information, available samples from a wide range of tissues should be collected from diseased animals including tissues from the respiratory organs and brain. In cattle and goats, milk samples should be collected from lactating animals. Samples of bulk milk can be used for surveillance activities in dairy farms, but care must be taken that milk from animals suffering from mastitis does not enter the milk tank and because a dilution effect might affect sensitivity.

Influenza A viruses (IAVs) are not considered to circulate widely in ruminants (unlike influenza D virus) and, therefore, tests targeting conserved portions of the influenza A genome could be used for screening purposes. Any sample resulting positive at generic AI tests should be then sequenced by WGS for the definition of genetic characteristics of the infecting virus and for the detection of zoonotic mutations.

4.6.6 | Surveillance in other domestic mammals

Other domestic mammals, such as alpacas, have been sporadically infected with currently circulating HPAI (clade 2.3.4.4b) viruses, although detailed pathology and clinical signs are not well documented (https://www.aphis.usda.gov/livestock-poultry-disease/avian/avian-influenza/hpai-detections/mammals/highly-pathogenic-avian).

In May 2024, the United States Department of Agriculture (USDA) announced the first detection of HPAI A(H5N1) in alpacas on a farm in Idaho. Four out of 18 alpacas on the farm tested positive for the A(H5N1) virus, and no alpaca deaths were reported. The alpacas were in close contact with infected poultry on the same farm, which was subsequently depopulated. Genetic sequencing showed that the virus infecting the alpacas (B3.13 genotype) matched the A(H5N1) strain currently circulating in the United States dairy cows and the virus found in birds on the affected farm.

Data about the susceptibility of wild rabbits are scarce. In the ongoing epidemic in North America different synanthropic mammalian species have been affected by HPAIVs, including desert cottontail rabbits, prairie voles and mouse spp. (USDA, online-b). Previous work has demonstrated that cottontail rabbits (*Sylvilagus* sp.) can be infected with clade 2.3.4.4 A(H5N1) HPAIV and will shed the virus (Root et al., 2018), but it is unclear if this extrapolates to the European rabbit (*Oryctolagus cuniculus*) or the European hare (*Lepus europaeus*).

Similar surveillance criteria to those used for domestic ruminants, could be applied to those other domestic mammals (e.g. alpacas, rabbits, horses, pigs):

- Surveillance should be triggered for other domestic mammals (**primarily found dead, sick animals** and, in the absence of those, sampling asymptomatic animals can be also considered based on decisions by local authorities):
  - with **epidemiological connections** to **infected poultry**, such as domestic mammals in contact with infected poultry (mixed farming environments). For example, this was the case for reported infected alpacas in the USA that were in close contact with infected poultry.[24]
  - with **epidemiological connections** to **infected wild birds**. Examples are domestic mammals in contact with confirmed wild bird cases found dead on the farm.
  - with **epidemiological connection** to **infected mammals**, such as domestic mammals with prior contact with infected mammals through the movement of animals, material and people.
- Surveillance should be triggered for domestic mammals showing **unexplained clinical signs** in **high-risk areas** and during **high-risk periods** of AI circulation.

The sampling approach is reported in Table 5.

[22] https://www.cidrap.umn.edu/avian-influenza-bird-flu/h5n1-traces-found-muscle-culled-dairy-cow-michigan-reports-3-more-affected.
[23] https://www.aphis.usda.gov/livestock-poultry-disease/avian/avian-influenza/hpai-detections/livestock/testing-and-science/meat-safety.
[24] https://www.aphis.usda.gov/livestock-poultry-disease/avian/avian-influenza/hpai-detections/mammals/highly-pathogenic-avian.

4.6.6.1 | *Type of sample and diagnostic test*

On domestic ruminants, limited reports on clinical presentation and tissue distribution in affected animals are available. Considering the limited information available, samples from a wide range of tissues should be collected from diseased animals including tissues from the respiratory organs and brain. This applies in particular to poorly studied species or novel hosts, where the virus tropism is unknown or not well understood. Tests targeting conserved portions of the influenza A genome could be used for screening purposes. Any sample resulting positive at generic AI tests should be then sequenced by WGS for the definition of genetic characteristics of the infecting virus and for the detection of zoonotic mutations. Specifically for pigs, for positive samples for type A influenza, additional molecular diagnostic testing to differentiate AIV from seasonal or swine influenza viruses that may be co-circulating, should be performed. WGS by appropriate methods should be used to identify reassortment events with other animal or human influenza viruses that could potentially generate a pandemic virus.

4.6.7 | Surveillance in farmed fur mammals

Fur mammals, such as mink, foxes and raccoon dogs, are animal species susceptible to AIVs. In the wild, sporadic outbreaks have been reported, while the important epidemiological role is played by these species as they are farmed in high numbers and densities, in open cages and buildings where there may be easy contact with wild birds. Therefore, farmed fur mammals should be especially targeted for sampling (ENETWILD consortium et al., 2024a).

For fur mammals:

- Surveillance should be triggered for fur mammals (**found dead, sick or asymptomatic**) with **epidemiological connections** to **infected wild birds**. Examples include fur animals in contact with confirmed wild bird (or wild carnivores) cases found dead on the farm. Indeed, fur animals are generally farmed in cages in partially open buildings where (infected) wild birds may have access, as occurred in the outbreak in a mink farm in Spain (Agüero et al., 2023) and several introductions from wild birds/the environment were seeding the 2023 outbreak in Finland (Kareinen et al., 2024).
- Surveillance should be triggered for fur mammals (**found dead, sick or asymptomatic**) with **epidemiological connection** to **infected mammals**, such as fur animals having epidemiological connections with infected fur farms through movements of materials, people, etc.
- Surveillance should be triggered when there is increased mortality in fur mammals or showing **unexplained clinical signs** in **high-risk areas** and during **high-risk periods** of AI circulation. This includes fur mammals found dead or showing unexplained clinical signs and which are already screened for specific diseases as part of a surveillance campaign (such as SARS-CoV-2) (opportunistic surveillance). Previous outbreaks have indeed shown limited specific clinical signs with depression and loss of appetite over bloody snouts and hypersalivation (ENETWILD consortium et al., 2024a).
- Surveillance is also necessary when these animals are found (found dead, sick or asymptomatic) near infected poultry farms; however, it is not very likely that fur animals will be raised in combination with poultry farming. Animal feed could be another important virus source if animals are fed with raw poultry (or any other potentially infected animal product, if not properly processed to inactivate the virus, e.g. heating/cooking) by-products, which might expose them to AI infection (ENETWILD consortium et al., 2024a).

The sampling approach is reported in Table 5.

4.6.7.1 | *Type of sample and diagnostic test*

Animals commercially farmed for fur include several species such as the arctic (blue) fox (*Vulpes lagopus*), the red (silver) fox (*Vulpes vulpes*), fox crossbreeds, the mink (*Neogale vison*), the raccoon dog (*Nyctereutes procyonoides*) and others. In Europe, multiple reports of HPAI cases in fur farms have been reported since 2022 in Spain and Finland and these cases have involved mink, foxes and racoon dogs. In a comprehensive review of HPAI cases in Finland's fur farms, the organs more consistently showing pathological lesions have been the lungs followed by the brain and liver (Kareinen et al., 2024). In most farms, increased mortality was reported to be associated with the presence of clinical signs (lethargy, neurological signs, diarrhoea, rapid death) (Lindh et al., 2023). In a few outbreaks, mortality was the only sign reported, while, in some farms, mortality was within normal ranges. The collection of oropharyngeal and rectal swabs have proven to be effective for surveillance purposes in live animals and carcasses. In recently deceased or euthanised fur animals a set of tissues comprising lungs, brain, liver, intestine and spleen should be collected. Generic AI tests can be used for the detection of infection by AIVs in clinical specimens and tissues. Infections in mink by different influenza subtypes such as avian A(H5N1), A(H5N6), A(H9N2) and A(H10N4) as well as human/swine A(H1N1) and A(H3N2) viruses were reported worldwide. Therefore, molecular investigations should take into account the possible presence of different viruses in the positive samples and the selection of tests to detect influenza A should cover a wide range of animal and human viruses to avoid false negatives. Any sample resulting positive at generic AI tests should be sequenced by WGS for the definition of genetic characteristics of the infecting virus and for the detection of zoonotic mutations.

In general, diagnostic tools mentioned in the chapters above, including serology, should be validated on different animal species, environmental matrices and food matrices.

4.6.8 | Public health surveillance

4.6.8.1 | *Enhanced AI surveillance to identify human infections*

Monitoring and testing exposed persons to AI is covered in Section 4.7.1 and in other ECDC guidance documents (ECDC, 2022b, 2023a).

ECDC has published surveillance guidance documents with considerations on the monitoring of AI during both the influenza season and the interseasonal periods: 'Surveillance and targeted testing for the early detection of zoonotic influenza in humans during the winter period in the EU/EEA' (ECDC, 2024b) and 'Enhanced influenza surveillance to detect avian influenza virus infections in the EU/EEA during the interseasonal period', respectively (ECDC, 2024a). In summary:

- For routine influenza surveillance, ideally all sentinel, i.e. through the sampling of representative specimens from a known proportion of the population, influenza-positive specimens from both primary and secondary care sources should be typed and subtyped and also a subset of non-sentinel samples (ECDC, 2022a).

To identify human infections with AI viruses as quickly as possible during ongoing AI outbreaks in animals, enhanced surveillance should be considered in the context of the ongoing AI outbreaks in animals:

- People admitted to hospitals with respiratory symptoms or other symptoms compatible with AI virus infection should be asked about exposure to birds (wild birds or poultry) or other animals (dead or alive) in the 2 weeks before symptom onset or, if not available, before admission.
- Patients admitted to the hospital due to respiratory or other influenza-related symptoms should be considered for influenza A/B testing and subtyping according to clinical decision and their risk of exposure to infected animals. More extensive testing, including typing and subtyping of all influenza A-positive samples should be considered during the summer months.
- Testing for influenza virus of all hospitalised patients with unexplained viral encephalitis/meningoencephalitis in whom a causative agent cannot be identified should be considered. Typing and subtyping those patients who test positive for influenza A should then follow to rule out AI.
- While sustained human-to-human transmission has not been reported, vigilance is essential in detecting clusters or signs of limited transmission, particularly in high-exposure settings or during seasonal influenza peaks, when reassortment risk is higher.

During the winter months when seasonal influenza viruses will probably be extensively circulating in the population, a risk-based approach is suggested for influenza testing, typing and subtyping for AI virus that would need to be proportionate to the epidemiological situation and the capacities of reference laboratories (ECDC, 2024b).

In general, related to specimens from both primary and secondary care sources:

- Any laboratory-confirmed influenza A-positive sample that does not subtype as seasonal influenza virus (i.e. gives a negative result for both A(H3) and A(H1)pdm09, when subtyping has been attempted) should be tested specifically for the avian influenza H5 subtype. Those samples should be sent to the national influenza reference laboratories for further testing/confirmation. A(H5)-positive samples should also be sent to the WHO Collaborating Centre or WHO H5 Reference Laboratories for further characterisation.
- All A(H5)-positive samples should be sequenced and sequences submitted as soon as possible to GISAID, ENA and/or other public databases. Mutations associated with increased zoonotic potential and adaptation to humans should be closely monitored. Antiviral resistance testing and antigenic characterisation should also be performed as soon as possible.
- Timely notifications from all MS of AIV infections are central to pandemic preparedness and prevention. MS are encouraged to comply with EU and international regulations for reporting AI-confirmed cases.

Raising awareness among HCWs (including primary care workers) and communicating the epidemiological situation is important in order to not miss or delay the diagnosis of potential human cases. It should include consideration of specific enquires about animal exposure and advise to lower the threshold for testing/typing and subtyping influenza A-positive specimens according to the epidemiological situation. Clinicians should be educated on symptoms compatible with AI infections and testing of symptomatic persons with a history of exposure as proposed in the published ECDC guidance documents 'Investigation protocol of human cases of avian influenza virus infections in EU/EEA' (ECDC, 2023a) and 'Testing and detection of zoonotic influenza virus infections in humans in the EU/EEA, and occupational safety and health measures for those exposed at work' (ECDC, 2022b).

4.6.8.2 | *Types of samples and diagnostic tests*

The types of samples and diagnostic tests for testing and detection of zoonotic influenza virus infections in humans in the EU/EEA have been discussed in previous guidance documents from ECDC/EFSA/EU-OSHA (ECDC, 2022b) and the WHO (WHO, 2018). Due to the possibility of higher viral load in the conjunctiva, eye swabs should also be considered.

Testing and detection of zoonotic influenza can be carried out with nucleic acid amplification tests (NAAT) (such as PCR), enabling a highly sensitive and rapid direct molecular detection of the viral genes. The approach to diagnosis using real-time RT-PCR adopted in most laboratories is based primarily on targeting the M1 matrix gene, which is a standard target for the differentiation of type A and type B influenza viruses. As genetic sequences differ among various subtypes of zoonotic influenza viruses, it is necessary to obtain or design PCR primers and probes that will specifically detect the influenza subtype of interest. HA-based and NA-based NAATs can then be used for subtyping of the influenza virus. Sequencing can also be used for that purpose.

All samples from positive human cases (and at least a subset of positive animal cases should be sequenced, please refer to section 3.6.2 for more information on sampling). Due to the broad diversity of zoonotic influenza viruses, surveillance by genomic evaluation has become indispensable. The nucleotide-level resolution of in-depth WGS permits phylogenetic analysis and molecular epidemiological studies to provide a detailed understanding of an outbreak. Sequencing is the most robust method for distinguishing between zoonotic and seasonal strains. The availability and increasing use of WGS for routine diagnostics can support the identification of zoonotic transmission events, investigate any potential mammalian adaptation and provide information for epidemiological investigations. There are tools available that can help with the identification and analysis of viral genomes (Borges et al., 2018; GenoFLU, online; Santos et al., 2024). Generation of high-quality genomes from sequencing reads of seasonal flu can be made in the all-in-one suite INSAFLU (Borges et al., 2018; Santos et al., 2024) that has some support for avian influenza (A/H5N1). Various tools for the classification of genomes beyond subtypes also exist, such as GenoFLU (GenoFLU, online) and integrated functionalities of GISAID (Shu & McCauley, 2017).

Serological tests should not be used for initial detection and characterisation of a potential zoonotic event. However, serological methods are techniques that utilise standard laboratory equipment that can identify zoonotic influenza virus subtypes and measure HA-specific antibodies to the virus to answer different questions related to previous exposure or prevalence in specific populations. Nevertheless, serological tests have several limitations that need to be considered, such as the fact that cross-reactions can occur between different lineages within one subtype, or even among different subtypes. Moreover, the results obtained only provide information on historical exposure to zoonotic influenza viruses and do not provide viral genetic information, which is vital for evaluating the potential pandemic threat of strains.

4.6.9 | Animal and human/public health laboratory capabilities and capacities as a prerequisite for surveillance

4.6.9.1 | *Testing capacity*

Member States should ensure sufficient laboratory capacity to perform molecular testing. Rapid and affordable diagnostic tests for field use should also be ensured in low-income countries (EFSA and ECDC, 2024).

According to the latest ECDC survey on capacities and capabilities to detect and characterise zoonotic influenza viruses, public health (PH) laboratories in EU/EEA countries reported a substantial level of expertise, capability and capacity to detect avian and other zoonotic influenza viruses in human specimens, and the majority (69%) can also genetically characterise the viruses (ECDC, 2023b).

Reporting of human cases is covered in Section 4.6.1.

4.6.9.2 | *Sequencing capacity and genomic surveillance*

Laboratories must be equipped with or have access to genetic sequencing platforms, and the generation of the complete genomes of identified AIV should be part of the diagnostic process. Sequencing should be used to determine whether there are mutations indicating mammalian adaptation, as well as mutations related to reduced susceptibility to antivirals and those indicating antigenic divergence from the candidate vaccine viruses and the viruses included in the vaccines.

4.6.9.3 | *Capacity for antigenic characterisation*

Viruses from human cases and those causing outbreaks in mammals should also be antigenically characterised to demonstrate if they are antigenically distinct from previously circulating viruses and from the candidate vaccine viruses. If there is an indication from any genetic sequence of a virus detected in animals that there may be a change in antigenicity, the virus should also be tested phenotypically. Antigenic characterisation of AIV can be done with HI and neutralisation tests.

All human specimens should be sent to the WHO Collaborating Centre or WHO H5 Reference Laboratories for further characterisation (WHO, online). Results are feeding the strain selection process during the biannual WHO Vaccine Composition Meetings (VCMs) to select appropriate candidate vaccine viruses (CVVs).

The Food and Agriculture Organization of the United Nations (FAO), in collaboration with the WOAH through their joint initiative, the WOAH/FAO Network of Expertise on Animal Influenza (OFFLU), monitors genetic and antigenic data of emerging zoonotic influenza viruses detected in animals, particularly avian and swine strains. OFFLU's activities draw on the expertise of FAO and WOAH country offices, the WHO Collaborating Centres (WHO CCs), network laboratories, research programmes and global partners. Since 2011, FAO and WOAH have contributed to the WHO VCMs by providing crucial genetic and antigenic data on circulating avian and swine influenza viruses. This collaboration aids in assessing and updating CVVs for zoonotic influenza strains globally.

4.6.9.4 | *Phenotypic testing*

Phenotypic tests (e.g. airborne transmission capacity in mammals) on viruses with genetic characteristics indicating zoonotic potential should also be performed in laboratories with an appropriate level of biosafety (BSL-3 or higher).

4.6.9.5 | *BSL3 capacity*

Importantly, HPAIVs like A(H5N1), are currently classified as group 3 biological agents. Laboratories carrying out work involving such biological agents (e.g. virus isolation) must determine the relevant containment measures in order to minimise the risk of laboratory infection.

For more information on testing and detection of zoonotic influenza virus infections in humans in EU/EEA you can refer to previous ECDC guidance (ECDC, 2022b). For more information on testing algorithms for potential human cases, you can refer to the ECDC human case investigation protocol (ECDC, 2023a).

4.6.10 | Environmental surveillance including wastewater monitoring

Environmental surveillance can be a complementary tool in monitoring AI as it can detect the virus in various natural settings, such as bird habitats and wetlands. Testing environmental samples from natural water bodies may support tracking the presence and spread of the AI virus in wild and domestic animal populations, while bathing water testing could also be used for informing PH preventive measures (Tiwari et al., 2024). Adopting a One Health approach that integrates animal, human and environmental surveillance can enhance tracking and monitoring of AI virus circulation.

Within environmental surveillance, wastewater monitoring (WWM) can offer a cost-effective and non-invasive method that can provide complementary data which can be of interest for providing insights on AIV circulation, (e.g. dairy cattle farms in the USA) (Germeraad et al., 2020; Honein et al., 2024; Lee et al., 2024). The benefits of WWM systems are:

- cost-effective and broad coverage

WWM offers a cost-effective solution by sampling a wide range of potential virus sources through a single wastewater sample, covering large areas more efficiently than traditional surveillance methods.

- non-invasive monitoring

WWM relies on sampling wastewater to detect pathogens excreted by populations, rather than requiring direct biological sampling from individuals or animals (e.g. blood draws, nasal swabs).

- complementary to monitoring methods

WWM can complement traditional monitoring methods by providing additional data that offers a broader understanding of AI dynamics, identifying hotspots and tracking virus spread over time.

- monitoring of emergence of new strains or mutations.

WWM can help provide information on the emergence of new strains or mutations related to mammalian adaptation.

While WWM can be a valuable tool for monitoring AI, there are also several limitations, potentially reducing the effectiveness and reliability of WWM in certain contexts and compared with other targeted surveillance methods:

- limited connection of farmed/kept animal populations to WWM.

The value of WWM as an early warning tool is probably limited to populations and products directly connected to the sewage system and requires further investigation. Faeces and urine from farmed/domestically kept animals generally do not enter wastewater systems; instead, they remain in litter (e.g. poultry) or in faeces pits, later repurposed as fertiliser or processed (e.g. poultry manure is frequently incinerated). This limits the utility of WWM for the early detection of AI viruses during animal-to-animal transmission cycles. Similarly, faeces and urine of companion animals do not enter

wastewater systems. Elevated levels of AI virus in wastewater reported in several studies from the USA (Louis et al., 2024; Tisza et al., 2024) may have been influenced by dairy processing plant effluent, as these facilities are typically connected to wastewater systems. As milk, unlike meat, is delivered frequently, WWM can facilitate the early detection of infections within dairy production settings:

- low viral concentrations

AIV in bird faeces may be present in wastewater at very low concentrations, making detection difficult (Honein et al., 2024), especially when only a small proportion of the bird population is infected. Low viral load might lead to false-negative data, making it difficult to assess the presence or absence of AI. Further research is warranted to determine the sensitivity of wastewater systems in detecting AIV.

- interference from contaminants and other pathogens

Chemicals, organic matter and other pathogens in wastewater can interfere with the detection of AIV, potentially leading to inaccurate results or the need for additional testing methods.

- differentiation between seasonal and AI A subtypes

Current protocols often lack the ability to distinguish between different influenza A subtypes, which becomes particularly relevant during the winter season when seasonal IAVs are widely circulating. As a result, WWM data may be less informative during these periods.

- inconsistent viral shedding

Virus shedding varies by infection stage, strain and bird species, making it difficult to consistently rely on wastewater for AI monitoring and leading to gaps in data. Sporadic shedding might result in intermittent detection, leading to gaps in surveillance data.

- difficulty in source attribution

Even if AI is detected in wastewater, it can be challenging to determine the exact source of the virus. Wastewater systems collect effluent from various sources, making it difficult to pinpoint whether the virus originated from mammals, animal products or humans, complicating efforts to trace and control AIV outbreaks. The expansion of WWM systems to include a broader range of environmental and animal sources would enable more efficient tracking and monitoring of AI virus transmission. To date, there is no experience with the detection of human AI cases through WWM.

- limited temporal resolution

Wastewater surveillance provides only a snapshot of AI presence, with time lags between viral shedding, sampling and analysis, making it less effective for real-time monitoring and response.

Further information on OH outbreak investigations and management after detection of AI virus in wastewater or environmental samples is provided in the recently published ECDC/EFSA technical report (ECDC and EFSA, 2025).

As part of the European Commission's pandemic preparedness efforts, the Health Emergency Preparedness and Response Authority (HERA) is working on wastewater surveillance for PH in cooperation with the European Commission's Joint Research Centre (JRC) through two initiatives, the EU-Wastewater Observatory for Public Health and the Global Consortium for Wastewater and Environmental Surveillance for Public Health (GLOWACON) (EU-Wastewater Observatory for Public Health, online). In addition, the Joint Action EU-WISH (EU-Wastewater Integrated Surveillance for Public Health) has also been set up to work towards the implementation of wastewater surveillance for PH in the EU/EEA (EU-WISH, online).

4.7 | Preparedness, prevention and control measures to reduce the risk of zoonotic avian influenza

In this section a set of prevention, protection and preparedness measures to reduce the risk of zoonotic AI is proposed both for animal health and public health, categorised as measures at farm and occupational settings, measures for the general public and measures to be applied in the natural environment.

4.7.1 | Measures at farm and occupational settings with high exposure to animals

Preparedness for animal health threats can be divided into the following actions: prepare, prevent, detect, respond and recover. This section focuses on measures to prevent virus introduction in farmed mammals, such as fur animals and dairy cattle as an example, to minimise virus spread within and between farms when an outbreak is detected, as well as measures to increase preparedness to face possible epidemics of AI in mammal species (Gary et al., 2021). In Figures 18–20 as examples, a diagram of possible actions and related objectives for prevention, control and preparedness against AI spread in fur animal and cattle farms, as well as the related PH measures, are shown, respectively.

Additionally, this section addresses preventing human exposure to zoonotic AIVs and minimising the risk of reassortment events in farms.

4.7.1.1 | *Prevention: Limiting virus introduction to farms*

To prevent the introduction of pathogens, including HPAIV, to livestock farms such as cattle, fur animal farms, pigs, sheep, goats and poultry farms, comprehensive biosecurity recommendations are available from other sources and will not be covered in detail in this opinion (Dewulf & Immerseel, 2019; EFSA AHAW Panel, 2017; WOAH, 2024). Implementing biosecurity measures is complex and compliance is challenging for several reasons, leading to frequent breaches (Delanglez et al., 2024; Racicot et al., 2011). In the current situation of the spread of HPAIV between dairy farms in the USA, several breaches of biosecurity have been identified, potentially contributing to disease transmission (see Section 4.7.1.3) (USDA, 2024b, 2024c). For biosecurity programmes to be effective, regular training and audits are required to ensure full and consistent implementation. Furthermore, although the general principles of biosecurity apply to any farm, their on-farm implementation may call for a specific biosecurity plan based on the strengths, weaknesses and exposure profile specific to the farm type or even farm location. For example, the location of farms of susceptible species (e.g. poultry and fur animals) and the structure of breeding systems are important factors to be considered to increase the risk of AI introduction into the farm and of further spread within and between farms:

- high density of commercial farms of susceptible species (both poultry and farmed mammals), primarily in areas close to wetlands (high density of waterfowl);
- certain animal production systems with low biosecurity in such areas, and/or livestock species often moved along the production chain, which may facilitate the AIV spread (e.g. dairy cattle in the USA).

For fur animal farms, specific measures should be warranted to prevent wild bird access. This could be achieved by either using nets or by avoiding open housing systems to prevent wild birds from entering into contact directly or indirectly with fur animals and their feed. As a precautionary measure, avoiding feeding domestic animals with raw poultry meat or raw milk originating from areas with HPAI outbreaks is also recommended, since, as already highlighted (EFSA and ECDC, 2024), feeding practices can be a significant potential source of AI infection in animals. Feed of animal origin (swine, poultry, fur animal) may introduce influenza viruses to animal populations if not properly processed (heated). Wild bird contact can also be reduced by not keeping feed and other farm-derived organic material accessible. The risk of introduction of seasonal influenza viruses from humans (i.e. farm workers, veterinarians) to susceptible animal populations (e.g. mink) that will increase the risk of reassortment events between animal and human influenza viruses also needs to be highlighted.

4.7.1.2 | *Preparedness: Being ready to face possible outbreaks in mammals and protect farm personnel*

To effectively respond to an outbreak of AI in mammals, national authorities are encouraged to develop contingency plans tailored to this specific emergency.

The objectives for these preparedness activities should be:

- to have legal provisions in place to implement measures;
- to have a clear contingency plan in place and with a clear definition of actions, roles and timing;
- ensure clear role definition and commanding lines;
- ensure availability of human recourses needed;
- offer seasonal influenza vaccine to personnel according to national recommendations;
- ensure laboratory capacity, including capacity to scale up timely WGS;
- ensure surveillance capacity;
- ensure capacity for culling (if planned) and disposal of carcasses and animal products;
- disseminate knowledge to personnel to be involved in outbreak response;
- ensure collaboration and communication between PH, occupational safety and health and animal health national/regional/local authorities;
- have a communication plan for relevant target groups (e.g. farmers, citizens, hunters);
- develop a risk assessment model with an approach (PH, veterinary and environmental authorities);
- design information to be collected on exposed individuals in suspected animal outbreaks.

Besides, medium-term and long-term strategies should be an important part of the preparedness to prevent AI and should prioritise densely populated poultry areas, especially those near wetlands where waterfowl density is high: reducing the density of poultry farms, particularly those in high-risk areas or near wetlands, can help minimise disease spread. Adjustments to production systems – particularly for high-density outdoor operations and those involving frequent bird movement – are recommended to reduce AI transmission risks (EFSA and ECDC, 2024).

Public health agencies should be proactive and prepare to respond to potential human cases in facilities that experience outbreaks in animals. This preparation should include several key measures, including cooperation with occupational safety and health authorities on the implementation of occupational safety and health (OSH) measures at workplaces, the implementation of technical and organisational workplace measures, the distribution of PPE, training and education of PH teams in proper PPE usage and set up logistics for specimen collection, large-scale screening and laboratory testing to differentiate AI virus from seasonal respiratory viruses that may be needed. For workplaces, it is the employer's obligation to carry out a workplace risk assessment and set preventive measures in an appropriate way, including the use of PPE where technical and organisational measures are not sufficient to protect workers. Additionally, agencies should ensure the availability of antiviral treatment and develop standardised protocols for treatment or post-exposure prophylaxis (PEP). To raise awareness among farm workers (see below), PH and veterinary agencies should collaborate with the OSH authorities and the social partners from the sectors concerned to communicate the risks around AI to the exposed groups and share information about potential exposure to AI infected animals and the symptoms associated with AI infection in humans. In an occupational context, employers must arrange proper information sharing, consultation with workers or their representatives and training of workers. Information should also be distributed to health-care providers on the epidemiological situation, the symptoms associated with AI infection in humans and the importance of enquiring about the history of exposure. A One Health approach, involving collaboration with occupational health and safety authorities that considers the interconnection between humans, animals and the environment, including the working environment, is essential for a timely and coordinated response.

Simulation exercises are a valuable tool for familiarising PH, OSH and animal health personnel with contingency plans and testing their effectiveness.

4.7.1.3 | *Control: Limiting virus spread and ensuring virus control at the farm*

Quarantine and restrictions on the movement of live animals according to EU legislation should be enforced following an HPAI outbreak in farmed mammals in the EU. A complete ban on live animal movements would be recommended on farms with a confirmed HPAI outbreak. Animal movements should also be stopped from contact with herds until the tests are negative for the presence of HPAI. In general, reporting and keeping records of single animal movements is essential to be able to trace and confine potentially infected animals and the infection source. As from lessons learned from the outbreaks in dairy cattle in the USA, the complete mechanisms of disease transmission between farms are not fully investigated and understood, but there is little doubt that the movement of cattle and shared staff, vehicles and equipment have contributed to the extensive spread (EFSA, ECDC and EURL, 2024b).

Further measures to apply in infected mammal farms can include culling of infected animals or all susceptible animals on infected farms (if foreseen and feasible) and proper disposal of carcasses and animal products such as manure or milk (to be heat treated to inactivate the virus). In areas with HPAI-confirmed outbreaks, strict measures would be needed to prevent indirect spread between farms (Figures 18 and 19).

Reducing the density of farms is an important measure to limit further between-farm spread of HPAI as described also in Section 4.7.1.2. This is considered one of the long-term measures to prevent HPAI outbreaks in poultry (EFSA and ECDC, 2024).

In HPAI outbreaks in poultry the infectious period between infection of the flock and implementation of disease control measures can be estimated (Hobbelen et al., 2020). During this period before confirmation of diagnosis, the virus levels in poultry houses are likely to be high with a risk of unprotected exposure of farm workers. To minimise this risk, it is recommended to have preparedness on the farm to initiate specific workplace prevention measures including the use of PPE when needed when HPAI could be suspected, before the confirmation of diagnosis, and the threshold for this should be low, e.g. already if a small rise in mortality in the flock. The same could be applied in other settings with a high risk of infection, like for example on fur farms.

A thorough epidemiological investigation and contact tracing should be carried out, aiming at identifying the source of introduction and potential further spread. More information on One Health investigations can be found in the ECDC/EFSA guidance (ECDC and EFSA, 2025). The recent experience of HPAI on cattle farms in the USA shows that there is a high risk of undisclosed infected contact farms that need to be identified to implement measures as above. For example, weekly monitoring of bulk milk samples before moving the animals could be an effective surveillance option to prevent further virus spread in high-risk areas and periods, without having to test each individual animal, which is also part of the HPAI dairy herd status programme in the USA.[25]

Several measures have been proposed within a PH response to outbreaks and potential human case(s) of AI infection in the ECDC/EFSA technical report 'Drivers for a pandemic due to avian influenza and options for One Health mitigation measures' (EFSA and ECDC, 2024) and the 'Investigation protocol for human exposures and cases of avian influenza in the EU/EEA' (ECDC, 2023a) and are discussed in detail below (Figure 20).

[25]https://www.aphis.usda.gov/livestock-poultry-disease/avian/avian-influenza/hpai-detections/livestock.

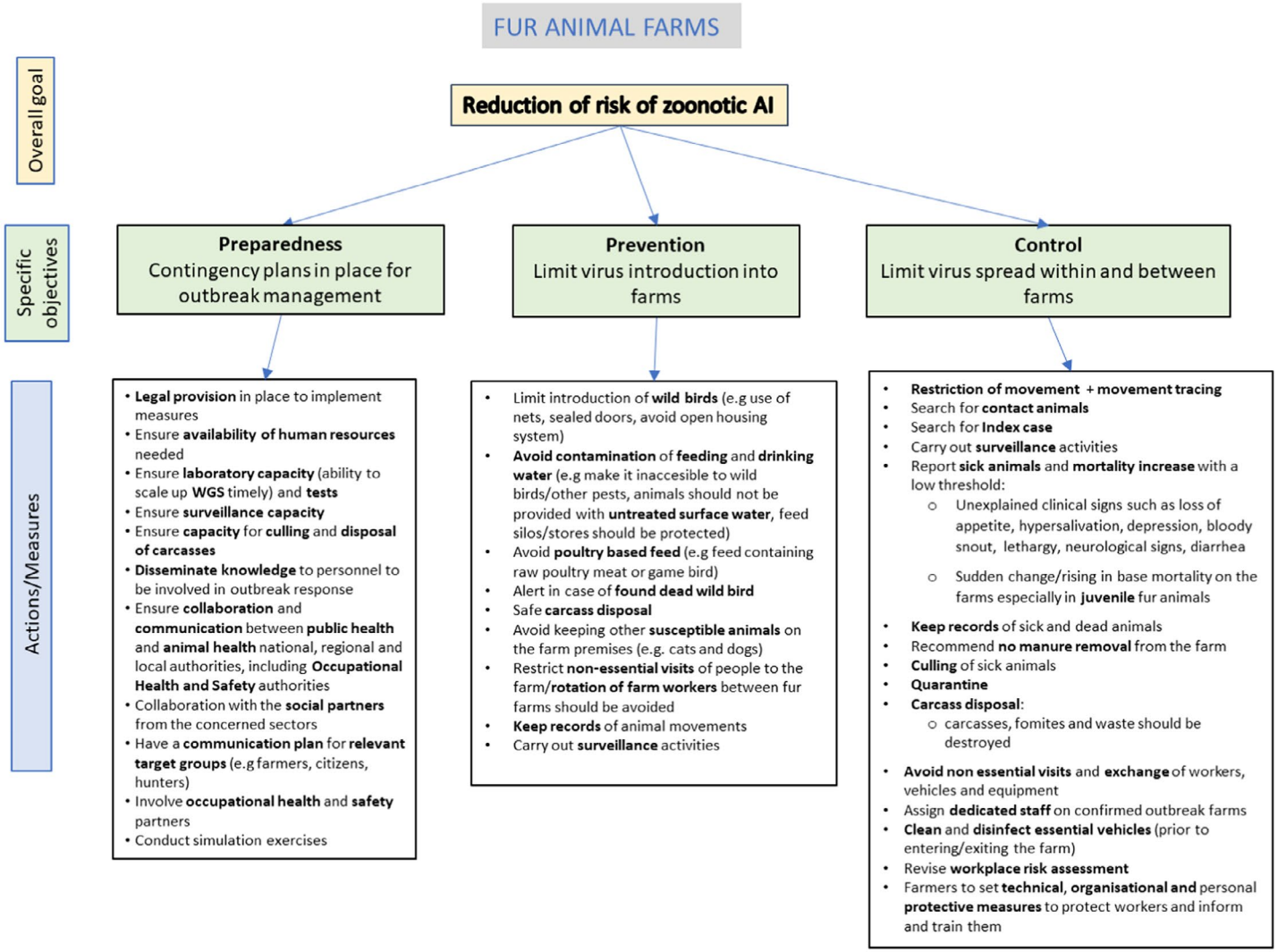

FIGURE 18 Diagram showing goals, objectives and possible related actions about prevention, control and preparedness measures to reduce the risk of AI spread from infected animals to and within fur animal farms.

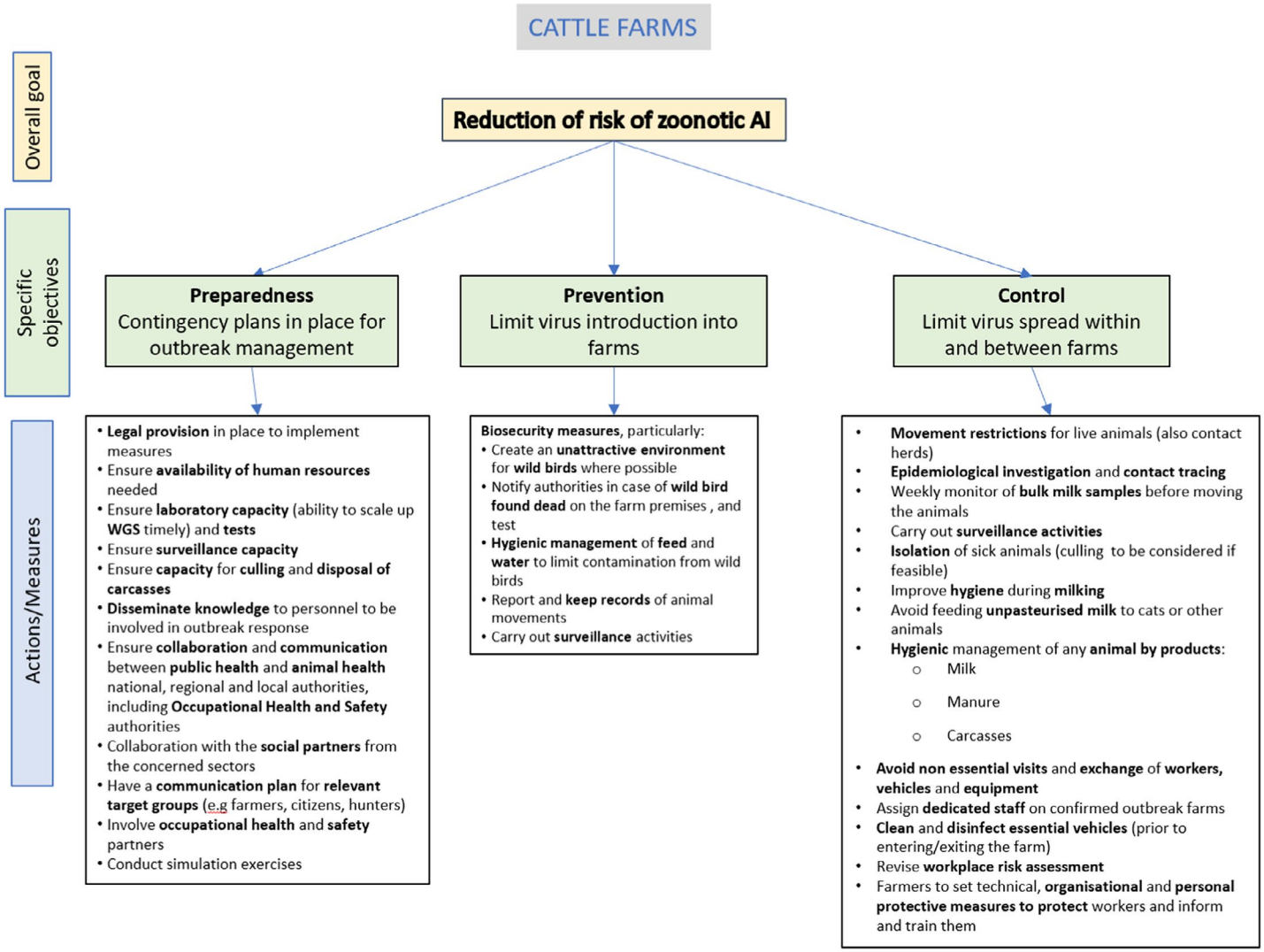

FIGURE 19 Diagram showing goals, objectives and possible actions related to prevention, control and preparedness measures to reduce the risk of AI spread from infected animals to and within cattle farms.

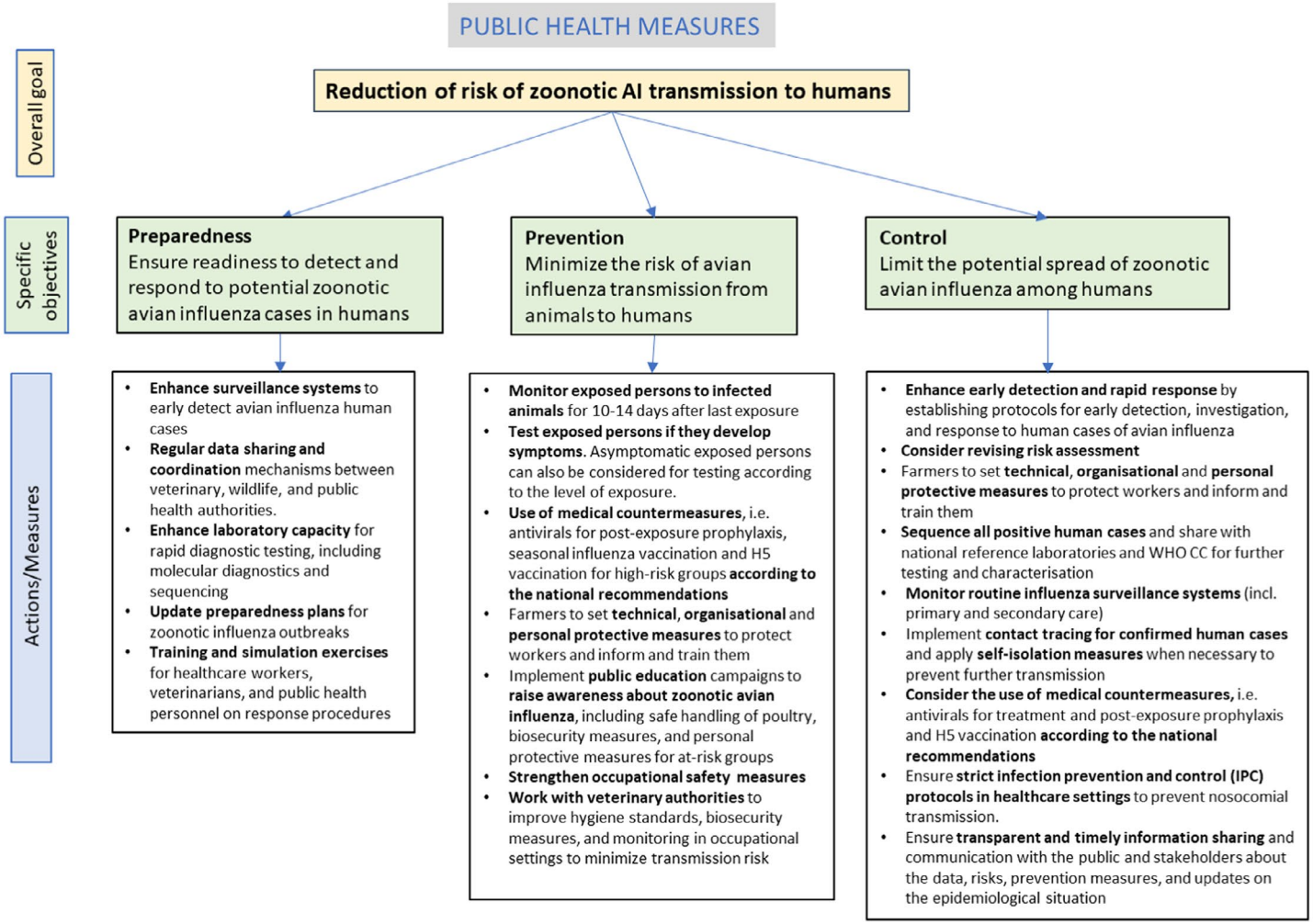

FIGURE 20 Diagram showing goal, objectives and possible actions related to prevention, control and preparedness measures to reduce the risk of AI transmission to humans in animal farms.

4.7.1.4 | *Monitoring and testing exposed people to animals infected with avian influenza*

ECDC has published an 'Investigation protocol for human exposures and cases of avian influenza in the EU/EEA' that sets out measures for the follow-up and management of individuals exposed to infected animals and human cases of AI, and for the PH management of possible and confirmed human cases of AI (ECDC, 2023a). People exposed to infected animals should be followed up to identify early transmission from animals to humans, as well as between humans.

The protocol recommends that follow-up should last between 10 and 14 days after the last exposure. This could involve active follow-up, where health authorities contact the individuals daily or frequently to check for the onset of symptoms, or passive follow-up, which might include daily self-checks and reporting of health status.

If individuals develop symptoms, which could range from fever, conjunctivitis and diarrhoea to respiratory, neurological or other atypical symptoms, immediate testing should be undertaken and the individuals should be isolated. Those who have been in close contact with confirmed human cases should also be tested and monitored to prevent further spread and to track transmission. Testing of asymptomatic individuals who have been exposed may be conducted on a case-by-case basis, taking into account the level of exposure. Serological testing can be applied to evaluate the seroconversion upon exposure and support the overall risk assessment for zoonotic transmission.

Certain activities and occupations have an increased level of exposure to animals and therefore risk of infection with AI. ECDC has published documents to assist the categorisation and assessment of different levels of exposure (ECDC, 2022b), where different activities/occupations according to the type, location, duration of exposure and potential viral load to which the individual has exposure are described (Table C.1 – updated, Annex C). In addition to the aspects covered in the aforementioned documents, several recent studies have shown that the viral load in the raw milk of infected cows can be high, making raw milk a potential source of transmission to other mammals, including humans (Caserta et al., 2024; Kozlov, 2024; Le Sage, Campbell, et al., 2024; NIH, online). Ocular exposure is a possible route of transmission, as the conjunctival route can serve as an entry portal for HPAIVs, harbouring α2,3-linked sialic acid (avian-type receptor) (Belser et al., 2018; Fouchier et al., 2004).

4.7.1.5 | *Occupational safety and health considerations*

Infections with zoonotic influenza have been observed in certain occupational settings, particularly those involving contact with infected animals or contaminated environments (EU-OSHA, 2019). Following an outbreak, it is essential to inform and consult national OSH authorities. AIVs, such as H5N1, H7N7 and H7N9, are currently classified as group 3 biological agents according to the Biological Agents Directive (Annex III Biological Agents Directive) (EU-OSHA, online-b), which requires employers to set appropriate measures tailored to the risk group and maintain records of workers exposed to these viruses.

A comprehensive body of legislation sets out the obligations of employers and the measures to be taken to protect workers (EU-OSHA, online-a, online-b). At the EU level, minimum requirements are outlined in the Biological Agents Directive (EU-OSHA, online-b) and must be transposed into national legislation by each MS. More detailed or stricter measures may be set in the MSs. There may also be codes of practice in place, which provide advice for specific sectors or for specific occupations, such as for example the technical rules for biological agents in Germany (BAuA, 2007b). Information may also be available for employers to support them in their duties (INRS, 2017). According to Article 3 or the Biological Agents EU Directive (EU-OSHA, online-b), employers are responsible for implementing appropriate preventive measures following a workplace risk assessment that needs to be updated regularly and in case of changes. For any activity likely to involve a risk of exposure, the nature, degree and duration of workers' exposure must be determined in order to make it possible to assess any risk to the worker health or safety and to lay down the measures to be taken (EU-OSHA, online-b). Based on this, employers have to set technical, organisational, maintenance and hygiene measures to prevent worker infection and protect workers' safety and health following a hierarchy of prevention that prioritises collective over personal protective measures. Employers need to consult workers or their representatives on the measures taken.

In occupational settings, such as agriculture, veterinary practices and laboratories, comprehensive health and safety measures are critical to protecting workers from zoonotic influenza, particularly during outbreaks and when handling potentially infected animals. Examples are outlined below, particularly for agricultural settings and in animal farming, although some principles (such as the protection of vulnerable groups) apply to all sectors and occupations. Employers must consult workers or their representatives in particular on the risk assessment and the prevention measures (Article 12 Biological Agents Directive and Article 11 Framework Directive 89/391) (EU-OSHA, online-a, online-b), as well as provide training.

Technical measures

These include the following:

- Avoidance/reduction of aerosols and dusts (for instance ventilation and air-conditioning systems (incl. appropriate maintenance), enclosed driver cabins on agricultural vehicles and proper ventilation of cabins of lifting and transport vehicles, optimising ventilation) in particular when handling dusty, organic materials in large quantities or when cleaning. Culling operations, in particular poultry, are also likely to generate a lot of dust (BAuA, 2007a).

- Easy-to-clean surfaces on floors, walls and work equipment (e.g. machines, equipment, production facilities) in the working area.
- Cleaning should be done in a way that dust and aerosols are minimised, for example by:

  – cleaning with a gentle water jet instead of high-pressure cleaning;
  – softening before wet cleaning;
  – cleaning with a vacuum cleaner fitted with a suitable filter;
  – wet cleaning instead of sweeping or blowing with compressed air.

- Means for safe collection, storage and disposal of waste by workers should be provided, including the use of secure and identifiable containers, after suitable treatment where appropriate (Article 6 Biological Agents Directive); this includes a validated inactivation process for the safe disposal of animal carcasses on site or off site (Annex V Biological Agents Directive) (EU-OSHA, online-b).

Organisational measures

Measures should be taken to ensure that activities or procedures leading to the release of biological agents do not result in worker exposure in neighbouring areas (e.g. staggered scheduling of activities or physical separation of areas). Access should be limited to those persons who have to work in the area, particularly in cases of outbreaks.

Separation of contaminated and clean areas: Employers should ensure separation between potentially contaminated and clean areas ('black/white areas') to prevent cross-contamination. This is particularly important in agricultural settings, such as poultry establishments and in waste management, for example when animal waste is disposed of, particularly when culling is planned. For instance, domestic areas on farms and housing areas for seasonal and temporary workers should be protected from contamination (e.g. preventing exposure through contaminated work clothing).

Decontamination facilities: Workers must be provided with appropriate and adequate washing and toilet facilities, which may include eye washes and/or skin antiseptics (Article 8.1(c) Biological Agents Directive) (EU-OSHA, online-b); (liquid soap, disposable towels and hand disinfectants, where appropriate). Where advised and in particular for outbreaks, workers should be able to shower at the worksite or at nearby decontamination stations at the end of their shifts. Employers should promote good hand hygiene practices. These practices help prevent infection spread and should be emphasised through training and awareness programmes. They should ensure that workers do not eat or drink in working areas where there is a risk of contamination (Article 8.1(a) Biological Agents Directive) (EU-OSHA, online-b).

Personal protective equipment (PPE) and work clothing

Workers must be provided by the employer with appropriate protective clothing or other appropriate special clothing. Working clothes and protective equipment must be removed before leaving the working area. Separate storage must be provided for work and street clothing (Article 8.1(b) Biological Agents Directive). Contaminated work clothing and PPE must be cleaned or disposed of by the employer (Article 8(2) Biological Agents Directive) (EU-OSHA, online-b).

Employers must provide appropriate PPE, including respiratory and eye protection (Article 14(3) Biological Agents Directive) (EU-OSHA, online-b), especially in environments where aerosols or dust may be generated. Workers must be trained in the correct use, storage and disposal of PPE. Employers must ensure that any necessary protective equipment is properly stored in a well-defined place, checked and cleaned if possible before and after each use, is repaired when defective, or is replaced before further use (Article 8(d) Biological Agents Directive) (EU-OSHA, online-b). Considering recent findings of viable AI viruses in raw milk, additional protection for the eyes should be considered in environments where aerosols or dust could be generated. Employers should assess and adjust PPE requirements accordingly.

Special consideration for vulnerable workers

Employers should include in their risk assessment workers who may be vulnerable because of their lack of experience or specific health risks and set specific measures to protect them. This includes pregnant or breastfeeding workers, young workers, workers with pre-existing health conditions and inexperienced workers. According to the Young Workers Directive (94/33/EC) of 22 June 1994 on the protection of young people at work (Article 7(2) and Annex)[26] with biological agents of group 3, such as the avian flu strands considered in this guidance, should be prohibited for young workers, with the exception of activities authorised by law at national level for vocational training, if protection of their safety and health is ensured by the fact that the work is performed under the supervision of a competent person. Employers should provide tailored training for vulnerable groups (Council Directive 92/85/EC, Article 4, Annex I).[27]

Migrant and temporary workers may be more at risk due to lack of experience, limited access to information or challenging working or housing conditions, in particular in crowded housing areas on site. Employers should ensure that these

[26]Council Directive 94/33/EC of 22 June 1994 on the protection of young people at work OJ L 216, 20.8.1994, p. 12–20.

[27]Council Directive 92/85/EEC of 19 October 1992 on introducing measures to encourage improvements in the safety and health at work of pregnant workers and workers who have recently given birth or are breastfeeding (10th Individual Directive within the meaning of Article 16(1) of Directive 89/391/EEC). OJ L 348, 28.11.1992, p. 1–7.

workers are included in their workplace risk assessment and are fully informed and trained in all issues related to health and safety at work, with special attention to language barriers or other communication challenges.

Specific measures during outbreak response

Employers need to set stricter measures specific to the case of outbreaks, to avoid infections with a group 3 biological agent. These can include the following:

- During outbreaks, animal culling and high-risk operations, access to contaminated areas should be restricted to essential personnel only. Proper decontamination of workers, their clothing, tools and machinery is crucial, as is the safe removal and disposal of PPE and contaminated waste, including animal waste.
- Strict decontamination procedures should be in place for handling tools, machinery and workers' clothing. Waste storage and disposal should be handled in closed containers, with proper transport of potentially contaminated materials. Employers should provide appropriate facilities for these activities.
- Employers should implement enhanced ventilation in work areas to reduce the risk of airborne transmission. Additional preventive measures should also be in place to avoid the generation of aerosols and dust, which can facilitate the spread of the virus.

Preparedness and worker training

Employers must draw up plans to deal with accidents involving biological agents (Article 6(f) Biological Agents Directive) (EU-OSHA, online-b). They shall also, when requested, make available to the competent authority appropriate information on an emergency plan for the protection of workers from exposure to a group 3 biological agent such as the zoonotic influenza strands in question that might result from a loss of physical containment (Article 7(f) Biological Agents Directive) (EU-OSHA, online-b). Workers should receive continuous training on safe working practices, including the correct use of PPE, hygiene measures and emergency procedures. Employers must ensure that workers and/or any workers' representatives in the undertaking or establishment receive sufficient and appropriate training, on the basis of all available information, in particular in the form of information and instructions, concerning: (i) potential risks to health; (ii) precautions to be taken to prevent exposure; (iii) hygiene requirements; (iv) wearing and use of protective equipment and clothing; and (v) steps to be taken by workers in case of incidents and to prevent incidents. The training shall be given at the beginning of work involving contact with biological agents, adapted to take account of new or changed risks, in particular in case of outbreaks and culling, and repeated periodically if necessary (Article 9 Biological Agents Directive) (EU-OSHA, online-b).

Occupational health surveillance and record-keeping

Employers should seek advice from occupational health services and/or occupational physicians to set out appropriate health surveillance measures, where available, or consult OSH authorities or sectoral organisations on the national rules that are in place. The doctor and/or the authority responsible for the health surveillance of workers exposed to biological agents must be familiar with the exposure conditions or circumstances of each worker and propose any protective or preventive measures to be taken with respect to any individual worker. Health surveillance of workers must be carried out in accordance with the principles and practices of occupational medicine (Annex IV of Biological Agents Directive) (EU-OSHA, online-b). It must include at least the following measures:

- keeping records of a worker's medical and occupational history;
- a personalised assessment of the worker's state of health;
- when appropriate, biological monitoring, as well as detection of early and reversible effects.

In cases in which health surveillance is carried out, an individual medical record shall be kept for at least 10 years following the end of exposure, in accordance with national laws and practice (Article 14.4 Biological Agents Directive) (EU-OSHA, online-b). If a worker is found to be suffering from an infection and/or illness that is suspected to be the result of exposure, the doctor or authority responsible for health surveillance of workers shall offer such surveillance to other workers who have been similarly exposed. Confidentiality of sensitive medical data must be respected at all times.

Employers shall keep a list of workers exposed to the zoonotic influenza viruses classified as group 3 biological agents, indicating the type of work done and, whenever possible, the biological agent to which they have been exposed, as well as records of exposures, accidents and incidents, as appropriate (Article 11 Biological Agents Directive) (EU-OSHA, online-b). The doctor and/or the competent authority for health and safety at work who carry out health surveillance, and any other person responsible for health and safety at work, shall have access to the list. Each worker shall have access to the information on the list which relates to him/her personally (Article 10(4) Biological Agents Directive).

The details of occupational health surveillance, record-keeping, worker's consent in an occupational context and follow-up should be discussed with national OSH authorities.

If workers are advised to self-isolate because of an infection or a suspected infection, employers should provide clear procedures, including the duration of isolation, health surveillance required, and support during this period. Workers should be able to follow PH recommendations and return to work safely after isolation.

Further information about occupational health and safety measures for those exposed at work can be found at the ECDC/EFSA/EU-OSHA guidance: https://www.ecdc.europa.eu/en/publications-data/zoonotic-influenza-virus-infections-humans-testing-and-detection.

4.7.1.6 | *Awareness raising for personnel involved in the agricultural sector*

All individuals within the agricultural community, such as employers, farmers, agricultural workers, animal transporters, slaughterhouse personnel and veterinarians should be provided with regular information and reminders about how AI spreads among animals and how to prevent infections, including appropriate protective measures and proper use of PPE (EFSA and ECDC, 2024). Information about symptoms of avian influenza A virus infection in humans, along with guidance on how to seek medical care if symptoms appear, should be made available in multiple languages and in a format that they understand (in particular for migrant or seasonal workers).

Employers should consider providing written instructions at the workplace and, if appropriate, display notices which shall, as a minimum, include the procedure to be followed in case of a serious accident or incident involving A(H5) viruses and develop emergency procedures and inform workers of any serious incident or accident and the measures taken or to be taken. Workers should be informed to whom they report any incidents or accidents to (Article 10 Biological Agents Directive). For a successful communication campaign, cooperation with labour and OSH authorities and social partners from the concerned sectors is essential.

Countries need to develop effective risk communication and community engagement strategies to ensure that the public and relevant stakeholders are aware of the situation, the risks associated with AI, and the measures being taken to prevent its spread, as has been recommended earlier (ECDC, 2022b).

4.7.1.7 | *Vaccination of animals*

Vaccination against HPAI can be a highly useful tool to prevent and control HPAI spread when integrated into a broader strategy of disease prevention and control (EFSA AHAW Panel, 2023, 2024; EFSA and ECDC, 2024). Different vaccines against HPAIVs are available (EFSA AHAW Panel, 2023). Also, experiments are running in the USA to develop an AI vaccine to be used in cattle.[28] The main key points defining the usefulness of vaccination for AI in poultry are:

- reduction in virus circulation: when effectively implemented, vaccination can significantly reduce the circulation of the AI virus among poultry populations. This helps in lowering the overall viral load in the environment, reducing the chances of outbreaks and subsequent spread;
- decrease in human exposure: by controlling HPAI in poultry, vaccination can indirectly reduce the risk of human exposure to the virus, which is particularly important for strains that have the potential to jump to humans;
- complementary to other measures: vaccination is not a stand-alone solution but should complement other measures already in place in poultry and wild birds such as biosecurity, early detection and surveillance. This integrated approach strengthens the overall effectiveness of prevention and control;
- prevention of economic losses and welfare impair: outbreaks of AI can be devastating for the poultry industry, leading to significant economic losses due to culling and trade restrictions; also, the animal welfare of infected animals is impaired by the clinical signs due to the infection, and by the specific control and prevention measures applied (e.g. indoor confinement of poultry raised outdoor during high-risk period of virus introduction). Vaccination helps to mitigate these losses by preventing large outbreaks.

When implementing vaccination against HPAI in poultry, different challenges and limitations should be considered, particularly:

- Correlate of protection: the lack of a reliable immunity threshold indicating protection and the lack of knowledge of the duration of protective immunity in vaccinated poultry hamper the planning and implementation of effective vaccination campaigns.
- Antigenic matching: the effectiveness of a vaccine depends on how closely it matches the circulating strains of the virus. The latest OFFLU report summarising the antigenic properties of circulating viruses can be found here (OFFLU, 2024). Continuous surveillance is required to monitor the virus's evolution and ensure that vaccines remain effective.
- Risk of vaccine-induced mutants: inadequate or improper vaccination can lead to the emergence of vaccine-induced viral mutants, which may be more virulent or resistant, complicating eradication efforts. Potential vaccine escape mutants that may emerge during vaccination need to also be taken into consideration and the risk of silent circulation is dependent on the surveillance plan in vaccinated animals.

[28] https://www.unmc.edu/healthsecurity/transmission/2024/04/24/usda-assesses-vaccine-to-protect-cattle-from-bird-flu-virus/.

Due to the above, the impact of vaccination on reducing the risk of human exposure to AI is still under investigation, and there is a need for ongoing evaluation and research to fully understand its implications.

In summary, vaccination against AI is a valuable tool with the potential to reduce virus circulation, protect human health, and safeguard the welfare of the animals and the poultry industry. However, its success will depend on its proper implementation along with continuous surveillance, and integration with other preventive measures.

4.7.1.8 | *Antiviral drugs*

The use of antivirals for PEP and the treatment of AI is extensively discussed in previous ECDC documents (ECDC, 2023a; EFSA and ECDC, 2024). So far, mutations associated with reduced susceptibility of A(H5N1) viruses to available antiviral drugs authorised for use in humans have rarely been identified in the circulating strains in EU/EEA (EFSA, ECDC and EURL, 2024a). The emergence of resistance to oseltamivir in treated patients has already been reported (de Jong et al., 2005; Webby and Uyeki, 2024).

Treatment of humans infected with AIV should be initiated as soon as possible after the onset of symptoms. Several observational trials have shown that treatment of patients infected with H5N1 (no specific clades) with oseltamivir within 2 days of symptom onset is associated with better survival. However, even when treatment is initiated beyond the initial 48-h window, it has been demonstrated to reduce the mortality risk in severely ill patients (Muthuri et al., 2013). To date, there are no data available for the use of baloxavir marboxil in H5N1-infected patients. In vivo evaluation of additional antiviral treatments, including combinations of antivirals, should be a goal for preparedness purposes. Post-exposure prophylaxis can also be considered for persons exposed to infected animals and for contacts of probable or confirmed human cases.

In animals, antivirals against influenza virus infection are not authorised in the EU and their use is prohibited by the EU legislation (i.e. Article 4 of Delegated Regulation (EU) 2023/361). Moreover, the potential use of antivirals for influenza outbreaks in poultry or other farmed animals, particularly mammals, raises several concerns because it may contribute to the emergence of drug-resistant strains (Parry, 2005), making it not only more difficult to treat an individual infection but also potentially reducing the effectiveness of these drugs overall and limiting the available treatment as well as prophylaxis options during a potential pandemic.

4.7.1.9 | *Seasonal influenza vaccination of occupationally exposed persons to avian influenza*

Individuals who are at risk of exposure to AIV-infected animals through their work can be offered immunisation against seasonal influenza to minimise the risk of reassortment between avian and human seasonal influenza strains and to potentially reduce the challenge of distinguishing between seasonal and AIV infections in these individuals. This would in general apply to occupational groups before the influenza season, although it needs to be noted that cross-protective benefits (against AIVs) are probably small. It is important to combine vaccination with comprehensive preventive strategies (e.g. offering low threshold testing for seasonal and AIV and implementing other preventive measures, as necessary). Specific vaccination recommendations are the responsibility of national authorities.

The implementation of seasonal influenza vaccination in high-risk settings for AI could serve as a valuable opportunity to identify barriers to vaccination, evaluate the effectiveness of immunisation campaigns and enhance key components of pandemic preparedness plans.

4.7.1.10 | *AI a(H5) vaccination of exposed persons*

An adjuvanted zoonotic avian influenza A(H5) vaccine has been recently approved by the European Medicines Agency (EMA) (EMA, online). Several new vaccine platforms are being developed targeting AI in humans, including mRNA vaccines, aiming to allow a faster production timeline. The target groups for vaccination would primarily be individuals who are routinely, occupationally or otherwise exposed to animals, their secretions or contaminated environments, including poultry/fur farm workers, veterinarians, zookeepers, laboratory personnel and others.

It needs to be noted that, to date, there is still no evidence available on the vaccine's effectiveness and whether it will offer protection against severe disease, infection or onward transmission. Taking this into account and also considering that recent infections from A(H5N1) in occupationally exposed groups are mild, and that there have been no infections detected in humans in the EU/EEA so far, there is insufficient evidence to recommend zoonotic influenza vaccination in all EU/EEA countries. FAO/WHO/WOAH have also given a global perspective on 14 August 2024 (FAO, WHO and WOAH, 2024b). These considerations are based on the current epidemiological situation and risk assessment; preventive measures may need to be adapted if the risk level changes. The recommendations may change based on new evidence becoming available about the vaccines and the virus.

The decision of a MS on whether to offer the vaccination should be based on careful analysis of the epidemiological situation and consideration of vaccination objectives. If vaccination is considered, it should be seen as an additional precautionary measure complementing the main measures mentioned in this document (i.e. implementation of technical and organisational workplace measures and use of PPE as appropriate, use of seasonal influenza vaccination at the appropriate time of the year and according to national recommendations, follow-up of exposed persons and testing, use of antivirals for PEP and treatment according to national recommendations, as well as activities related to risk communication, training and education).

Before a vaccination is rolled out, it is important to ensure that the circulating strains are antigenically similar to the vaccine virus component. Those countries that choose to deploy AI vaccines in occupationally exposed individuals should consider assessing the benefits and risks of vaccination as part of a phase IV post-authorisation assessment. Vaccine deployment should be accompanied by monitoring of vaccine safety and uptake, as well as influenza surveillance including monitoring of virus characteristics. Given that vaccine effectiveness can only be measured in the context of a sufficiently large number of cases among exposed vaccinated and unvaccinated individuals, serological and in vitro studies evaluating humoral and T-cell adaptive immune responses are of value to understand the level and duration of antigen-specific immune responses. Furthermore, cohort study approaches could be applied to assess multiple factors impacting protection delivered by vaccination in occupationally exposed individuals.

A lower threshold for testing of asymptomatic vaccinated persons exposed to infected animals could be considered due to the uncertainties related to the effectiveness of the vaccine against infection and to ensure the detection of asymptomatic/mildly symptomatic cases. Surveillance and follow-up practices may need to be adapted in vaccinated occupationally exposed individuals as they may not become visibly symptomatic in case of infection. Monitoring of virus evolution will remain crucial to track any changes in the antigenicity of circulating strains compared with the ones that are included in the current vaccine formulation.

National legislation concerning OSH may vary across MSs. It is important that any vaccination strategy is adapted to the national legislation concerning workers and that national OSH authorities are involved in the development of the strategy, to ensure that national specificities are accounted for in the design of the strategy. According to EU legislation (EU-OSHA, online-b), when necessary, effective vaccines should be made available for those workers who are not already immune to the biological agent to which they are exposed or are likely to be exposed. If the workplace risk assessment reveals that there is a risk to the health and safety of workers due to their exposure to biological agents for which effective vaccines exist, their employers should offer them vaccination, according to national guidance. Workers should be informed of the benefits and drawbacks of both vaccination and non-vaccination, and vaccination must be offered free of charge. Vaccination should be carried out in accordance with national law and/or practice. Detailed information concerning occupational health surveillance is provided in Section 4.7.1.5.

4.7.2 | Measures for the general public

Measures for the general public on AI, as outlined in previous guidance documents from ECDC and EFSA (ECDC, 2023a; EFSA and ECDC, 2024), emphasise clear PH communication, awareness and practical prevention strategies. Public messaging should be timely, clear, transparent, and based on the latest evidence to counter misinformation and provide factual resources. Efforts to educate the public should include accessible information on zoonotic AI, modes of transmission and the importance of the issue. Information should be spread through diverse media channels, such as social media, radio, TV advertisements and posters, to reach various demographics. Educational content should cover the symptoms of AI in animals and humans and highlight the importance of seeking medical advice after potential exposure.

Encouraging good hygiene and sanitation practices is crucial, including regular handwashing with soap and water or the use of alcohol-based sanitisers, especially after contact with birds or contaminated surfaces. Safe handling of poultry and wild animals should be emphasised, such as wearing gloves, avoiding face contact and thoroughly washing hands and surfaces.

The public should also be advised to minimise exposure by avoiding risky behaviours. This includes staying away from sick or dead wild animals and understanding the risks of backyard poultry farming.

Reporting protocols should be clear to the public, including where and how to report sick or dead animals to local authorities and how to report suspected AI outbreaks to health and veterinary services. Individuals in contact with potentially infected animals who develop influenza-like symptoms should be encouraged to seek testing and treatment promptly. The use of antivirals for PEP or treatment should be considered in line with clinician guidance and national recommendations.

4.7.3 | Measures in the household

Susceptible species of companion animals such as cats, dogs and ferrets in households and with access to outdoor roaming have a high risk of being exposed to AI infection, e.g. by contact with dead or sick infected birds, other companion or feral animals, in particular in areas with a high density of wild birds and/or poultry farms where AI outbreaks are detected. Therefore, preventing companion animals from coming into contact with dead wild birds found outside (pets should not be left unattended outside in areas and periods during active outbreaks, but kept on a leash or, if possible, outdoor access might be avoided in that period), and reporting signs of illness, especially neurological or respiratory signs, in pets to veterinarians would be useful measures to avoid exposure of AI infection in the household (EFSA and ECDC, 2024).

In case of HPAI outbreaks in farmed mammals (e.g. cattle, goats), avoiding contact with companion animals living at the farm or in the neighbouring of the farm with those infected at the farm would be useful as well to prevent their infection. In addition, in areas where the virus is present, avoiding feeding companion animals with any raw meat from game birds

or poultry or colostrum or raw milk from cattle might also be considered as an additional and precautionary measure to prevent infection of pets.

There should be clear advice on keeping poultry separate from household spaces and on avoiding the consumption of unpasteurised raw milk and, as a precautionary measure, raw milk-derived products that have been produced in areas where AI outbreaks in dairy farms are reported. Research indicates that influenza viruses, such as A(H5N1), can remain viable in raw milk for a period of time depending on the storage temperature. Standard pasteurisation has been shown to effectively inactivate the virus, thus rendering commercial milk and products made from pasteurised milk safe for consumption. Avian influenza virus particles could potentially survive during the production process of dairy products made from raw milk, depending on the product and processing conditions used. Further research is needed to determine whether viable AIV can still be present in raw milk-derived products, and whether these could represent a source of infection for humans. Good food handling practices – such as cooking, cleaning, separating and chilling – should be promoted as sufficient household safety measures.

Although this has been observed in other mammals, there is no evidence that AI can be transmitted to humans through the consumption of contaminated food products. For poultry, the chance of meat from infected poultry or eggs from infected laying hens entering the food chain is extremely low because of the rapid onset of symptoms in poultry and the deriving safeguards in place (EFSA, ECDC and EURL, 2024b). For the outbreak in cattle in the USA, to date no virus had been detected in beef samples from infected herds with the exception of only one sample from a cull dairy cow (see Section 4.6.5.1). Viral presence in food is limited to foods derived from infected food-producing animals that are extremely unlikely to enter the food chain. Moreover, industrial pasteurisation and recommended cooking practices play a significant role or are effective in inactivating AIV in milk, meat, eggs and their products (EFSA, ECDC and EURL, 2024b).

4.7.4 | Measures for backyard poultry farms

In addition to the precautions for companion animals, specific measures should be considered for households involved in backyard poultry farming, particularly in areas affected by AI outbreaks. Exposed persons via backyard farming activities are common among AI-confirmed human cases and those populations that turn out seropositive in seroprevalence studies, conducted mainly in Asia. It is crucial to prevent direct contact between backyard poultry and wild birds, especially in areas and periods of high risk, as wild birds are often carriers of AI. During high-risk periods, poultry should be physically protected (e.g. housed or under secure netting) to limit exposure to wild bird populations. If animals, particularly poultry or other farm animals, show signs of illness or unusual behaviour in a backyard setting raising the suspicion of AI being present, owners should be aware of the actions that would be needed; the animals should be isolated from other healthy animals to prevent further spread and veterinary services should be promptly informed. Owners should also be made aware that sick animals should be handled with appropriate protective and hygiene measures (e.g. gloves, masks and dedicated clothing), and any animal deaths or suspected cases of illness should be reported to local authorities for proper testing and containment measures.

4.7.5 | Measures to be applied in wildlife (wild mammals)

As explained in this document, several spillover events of HPAIV to wild mammals have occurred and these may also lead to virus adaptation to mammals and the selection of potentially zoonotic strains. Therefore, the control of these events in wild mammals should be part of the preparedness, prevention and control strategies.

The management of HPAI outbreaks in wild mammals requires a comprehensive and interdisciplinary approach that integrates not only the monitoring of virus circulation, but also the implementation of control strategies in collaboration with official institutions at national and international scales and relevant stakeholders. Also, the great variability of epidemiological scenarios in which HPAI outbreaks can occur (e.g. wild environments, farms, zoos, etc.) strongly influences the control strategies to be followed in mammals.

The wildlife expert network of ENETwild discussed in detail the main strategies to prevent and control AI infection in wild mammals according to prevention, control and preparedness for free-ranging wildlife, captive wild mammals and zoo or rehabilitation centres for wildlife (ENETWILD consortium et al., 2024b), these are summarised below in Tables 6–8.

TABLE 6 Examples of prevention tools for different wild mammal categories.

	Biosecurity	Feed sources	Species separation	Landscape design
Free-ranging wildlife (incl. synanthropic wildlife)	Biosecurity for wildlife handlers	Carcass removal		To avoid or properly manage aspects that may increase density or chance of interface between birds and mammals: to avoid artificial water bodies, to limit habitat fragmentation, to manage waste disposal sites
Wild animal species kept in captivity	Biosecurity practices for animal handling, feeding, equipment/ workers	Consider safe feed sources	Avoid mixed farming (presence of poultry on mammal farm)	Avoid location of premises in proximity to wild birds gathering sites, wetlands, water bodies
Zoos/rescue centres	Quarantine, biosecurity practices for animal handling, equipment, PPE, spatial planning of animal premises	Consider safe feed sources	Avoid contact between mammals and HPAI bird reservoir species	Avoid locations in proximity to wild birds gathering sites, water bodies

TABLE 7 Examples of control practices for different wild mammal categories in case of outbreak.

	Identifying outbreak area	Biosecurity	Culling or isolation	Vaccination
Free-living wildlife, incl. synanthropic wildlife	Passive and active surveillance (diagnostic testing) in and beyond outbreak area	Compartmentalisation, use of PPE, if disinfectants are applied, to consider the environmental impact of product	In case of localised mammal-to-mammal transmission, focal culling to be considered (or isolation of sick animals, especially if of conservation value). Of limited value in marine mammals	
Wild animal species kept in captivity	Reporting of sick animals and mortality changes even with low threshold	Physical barriers, cleaning and disinfection of premises, equipment, vehicles, waste removal, fomites and attractants (e.g. feed), efficient rodent control, use of PPE	Culling of all or a subset of animals on the premises to stamp out the outbreak	
Zoos/rescue centres	Passive (and active) surveillance in and beyond affected enclosures. Testing of environmental samples could complement	Physical barriers, cleaning and disinfection of premises, equipment vehicles, removal of waste, fomites and attractants, efficient rodent control, use of PPE	If conservation value, proper isolation during attempted recovery	Ongoing in some zoos. Difficult to implement in rescue centres

4.7.5.1 | *Preparedness*

Surveillance and response to HPAI in wild mammals involves a complex network of stakeholders of diverse nature (i.e. various ministries, veterinary authorities, wildlife managers, farm workers and associations representing those who work in zoos and rehabilitation centres as well as the general public), it is essential to create and consolidate a network in 'time of peace' to ensure efficient communication, coordination and action in the surveillance or during an outbreak.

TABLE 8 Preparedness tools for different wild mammal categories.

	Stakeholders network	Legislation	Wildlife distribution	Predictive models
Free-living wildlife, including synanthropic wildlife	Hunters, wildlife managers, forestry ministry and veterinary authorities		Access to knowledge on distribution and abundance of wild birds and free-ranging mammal populations over time	Risk models for spillover events in wild mammals
Wild animal species kept in captivity	Farmworkers and owners, feed producers, ministry of agriculture and veterinary authorities, farm veterinarians	Legislation to streamline animal sacrifice	Captive wild animal farms (and biosecurity measures) with a number of animals housed, neighbouring poultry farms. Mapping of wild bird distribution, abundance and movements with respect to farms	Risk models for spillover events in wild mammals

TABLE 8 (Continued)

	Stakeholders network	Legislation	Wildlife distribution	Predictive models
Zoos/rescue centres	Workers, managers, zoo veterinarians, wildlife rescue centres managers, animal ambulance managers, veterinary authority		Registry of animals and species held in the structure	Risk models for spillover events in wild mammals

4.8 | Sources of uncertainty

The sources of uncertainty and limitations related to the mutation analysis (see Section 4.4) are linked to the following aspects (not in order of importance):

1. The analysis relies on available sequence data that may not be representative of the global virus population (Figure 21). The data are skewed towards mutations in (i) certain subtypes (i.e. HPAI and notifiable subtypes that represent a major issue for the poultry industry); (ii) in regions with more intense virologic and genomic surveillance; and (iii) in different hosts due to differences in surveillance of different mammalian and avian species. Therefore, mutations that occur in less monitored areas/subtypes/hosts may be underrepresented or entirely missed.

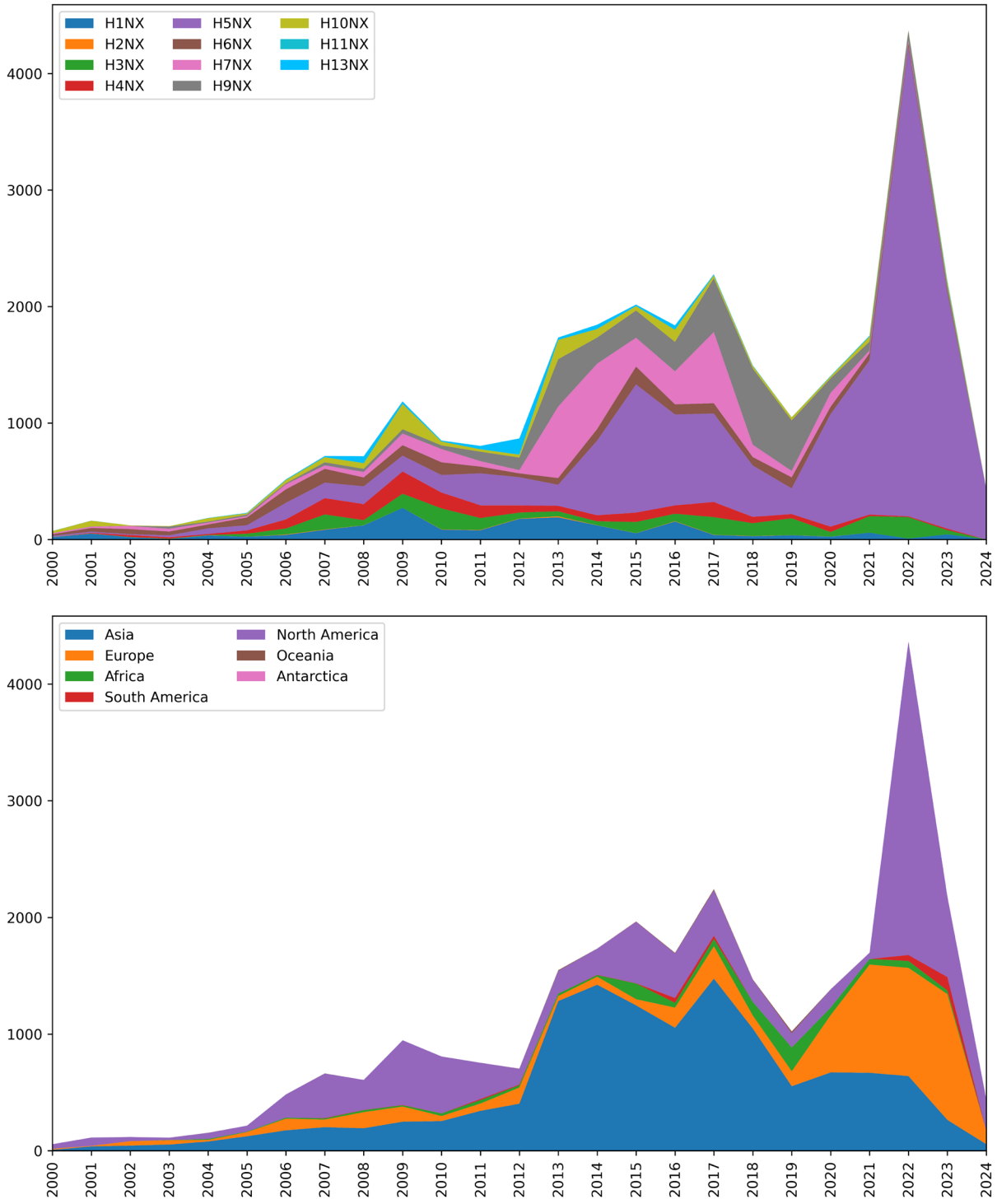

FIGURE 21 Number of sequences in the data set per year, subtypes (upper) and continent (lower).

All the analyses are clearly influenced by the availability of data. From the year 2000 to approximately 2007, the number of published complete genomes was notably low, which is reflected in the flat trend during this period. This suggests that the limited number of genomes available for analysis could significantly impact the overall conclusions drawn during these years.

2. Mutations observed at the genomic level do not always provide clear information about their functional effects. Even if a mutation has been associated with certain phenotypic traits in previous studies, it is not guaranteed to have the same effect in all viral or host contexts and that the host has acquired this trait. Many factors influence the effect of a mutation, including protein structure and interactions with other viral mutations or components. The mere presence of mutations connected to a zoonotic trait in the genome of AIVs does not directly imply significant viral adaptation to humans. Many mutations may not confer any selective advantage or may even be detrimental to the virus in certain circumstances. In addition, a mutation that appears advantageous may not be so in the presence of other mutations and vice versa. Gene interactions are difficult to predict based solely on

genetic sequences. Moreover, the presence of unknown mutations, whose effect has not been already demonstrated, cannot be excluded.

3. Because of the above, it should be noted that genomic sequencing of the viruses alone does not allow the prediction of their phenotypes because of the diverse gene constellations of the different AIV subtypes. In assessing the risk, data on findings of in vivo and in vitro experiments generated by multiple laboratories worldwide on each zoonotic threat should be collected.
4. Evidence of an association of a mutation to a specific phenotypic trait may be generated in experiments in a specific species (e.g. mouse, ferret) but will not necessarily result in the same phenotype in humans.
5. The genetic analysis as such does not account for the ecological context that can affect zoonotic risks, such as human and animal population density, species mobility and virus–host interactions. A genome-based assessment alone cannot predict how the viruses would behave according to different ecological and environmental aspects.
6. The genetic analysis does not consider genetic reassortment and deletion, truncation or insertion events, which can generate variants with new combinations of mutations and that may affect the virus properties and would not be predictable from this analysis. Any NA stalk deletion should signal a potential adaptive change of the neuraminidase.
7. Trait analysis has some important limitations. In our analysis the presence of a single mutation associated with a trait was sufficient to assign that trait to a virus strain. However, not all the mutations have the same impact on a trait (i.e. HA Q222L substitution can cause a switch in the receptor-binding preference from avian-type to human-type receptor, while other HA substitutions associated with the same trait may only increase the capacity of the virus to bind human-type receptors, although it continues to retain its binding preference for avian-type receptors). Moreover, the presence of one or multiple mutations associated with the same trait may have a different impact on the virus phenotype.
8. To increase the sensitivity of the analysis we have set a low threshold for considering a mutation as relevant (criteria in step 4, Section 3.1.5), for example by considering mutations observed in previous pandemic strains in the shortlist. For this reason, some mutations that were shortlisted and are shown in more detail in the graphs and figures may be actually less relevant for assessing the zoonotic potential of the virus.
9. There may be other phenotypic traits that have not been considered here that could have contributed to higher phenotype scores of pandemic isolates, potentially resulting in underestimating the impact of mutations that could distinguish pandemic viruses from other AI strains. Specifically, not all mutations affecting the HA stability have been included in the analysis. The mutation list should not be considered exhaustive.
10. Mutations that have been studied more extensively (have been subjected to more in vivo and in vitro studies), e.g. those in PB2, may be over-represented due to higher scores in step 3 (Section 3.1.4). Moreover, certain subtypes (i.e. H5, H1 or H7) have been studied more extensively than others and mutations identified from these studies in these subtypes may have no or a different effect on other subtypes.
11. The information about the phenotypic trait is available only for mutations that have been tested, meaning that a score of 0 in the original table may be due to either that the mutation has no effect on this trait or that the mutation effect on the trait was not tested and therefore no information on its effect is available (but it still appears as a 0).
12. Different reference virus sequences could be selected for extracting mutations that have been observed in previous pandemics. Currently, for the H1N1 1918 pandemic, a reference not originating from a virus from the beginning of the pandemic was used resulting in one mutation 432E in NA being included in the mutation analysis, but not necessarily relevant for increased zoonotic potential. By selecting mutations that are more frequently identified in mammals compared with birds, mutations that are linked to zoonotic potential may be missed if they have become established in widespread circulation in birds.

The sources of uncertainty related to the assessment of the surveillance (Section 4.6) are linked to the lack of information on species susceptibility and the epidemiological role of all domestic and wild species.

The sources of uncertainty related to the preparedness, prevention and control measures (Section 4.7) are linked to the possible lack of studies on the effectiveness of the different measures.

5 | CONCLUSIONS AND RELATED RECOMMENDATIONS

5.1 | Risk of AIV mutations towards adaptation to mammals

5.1.1 | Mutation analysis

- This analysis presented an updated list of mutations described in the literature associated with phenotypic traits, which may increase virus zoonotic potential or other effects linked to the adaptation of AIV to mammals.
- It also offers a list of mutations selected based on a score of evidence and expert opinions that deserve attention and should be monitored in the molecular analyses and genomic surveillance of AI. This list can be used by animal health, PH laboratories and researchers to guide future surveillance efforts.
- The 34 shortlisted mutations identified and associated with the following phenotypic traits are:

  - increasing mammalian specificity of virus attachment to receptor (HA:156A, HA:156V, HA:186D,221D; HA:186V; HA:221D; HA:222L; HA:224S),
  - increasing HA stability in mammal's environment (HA:222L),
  - increased activity of the viral polymerases in mammalian hosts (PA:356R; PA:552S; PA:85I; PA:97I; PB1-F2:66S; PB2:271A; PB2:292V; PB2:526R; PB2:588I; PB2:588V; PB2:591K; PB2:591R; PB2:627K; PB2:627V; PB2:631L; PB2:701N; PB2:702R),
  - evasion of innate immunity and counteraction of mammalian restriction factors (MP1:95K; NP:100I; NP:100V; NP:283P; NP:313V; NP:313Y; NP:52H; NP:52N),
  - disruption of the second sialic acid binding site (2SBS) in neuraminidase (NA:399R; NA:432E).

- In the retrospective analysis of thousands of sequences of IAVs exclusively of avian origin collected in mammals from 2000 to 2024, sporadic instances of viral subtypes with multiple mutations associated with different traits relevant to zoonotic evolution were observed. Notably, this includes A(H7N9), A(H3N8) and A(H5N6) viruses identified in Asia and A(H9N2) viruses detected in both Asia and Africa. The combination of five traits was identified only in 144 viruses of the H9N2 subtype identified in Asia and Africa between 2012 and 2023.
- Based on the available information, the worldwide increase in the number of mammalian (including human) infections associated with A(H5) clade 2.3.4.4b is probably mainly due to the epidemiological situation (high virus circulation in wild birds, high environmental contamination and increased exposure opportunity for both humans and mammals) rather than to specific genetic mutations. However, such infections can increase the likelihood of the emergence of adaptive mutations, reassortments and the accumulation of traits that increase pandemic potential.
- In EU/EEA, in the last 3 years, H5Nx viruses of clade 2.3.4.4b are those that have acquired the highest number (up to four) zoonotic traits, with the vast majority of them showing two or three traits.
- Adaptive changes in mammals occur in the genome of AIVs at different frequencies depending on the phenotypic trait involved. This is probably a consequence of the different impacts of certain categories of changes on viral fitness in the affected host.
- The increased activity of the viral polymerases in mammalian hosts, which may favour infection and viral replication of AIVs in mammalian species, as well as mutations associated with the ability to evade innate immunity and counteraction of mammalian restriction, appear to be the phenotypic traits that have been most frequently acquired by AIVs, among the five traits considered.
- In this analysis of sequences up to May 2024, viruses with at least one of the mutations in HA, involved in increasing receptor affinity towards human receptors and observed in previous pandemic events, have emerged mainly in Asia and Africa.
- Some of the viruses with an increased number of mutations that affect critical traits are low pathogenic viruses in poultry with a sustained or endemic circulation in these species in some countries (e.g. A(H9N2) and A(H3N8)).
- It is important to emphasise that the acquisition of mutations for adaptation to humans, as covered in this analysis (that did not include reassortment events), is thought to be a gradual process, and while amino acid substitutions are common, the accumulation of multiple mutations and phenotypic traits required to significantly increase zoonotic potential in a single host is considered to be an uncommon event. However, it is also true that the circulation of AIVs in regions with limited surveillance capacity, such as low-income countries, makes it challenging to track their evolutionary steps, potentially leading to gaps in our understanding of these processes.
- To date, only a few A(H9N2) viruses have been found to acquire the highest number of traits discussed in this analysis. It should also be noted that other genetic factors not included in this genetic analysis, such as reassortment, could dramatically affect the pandemic potential of AIVs.
- The described mutations and traits in this document warrant particular attention due to their possible influence on the zoonotic potential of AIVs based on the available literature and expert opinion, but it is also important to consider that further mutations or additional phenotypic traits for which limited evidence exist, that are poorly or not investigated to date, may be demonstrated in future studies to be relevant.
- The large amount of genomic data and related information compiled in this current work and their sharing with the public can represent an important step towards a more refined analysis, which, nevertheless, would require more time beyond the deadline for this opinion. The list of mutations needs to be continuously updated as more studies become available and new mutations may emerge.

Recommendations

Future studies and data harmonisation

- It is recommended to set up and invest in systems for the timely characterisation of emerging viruses, focusing on evaluating the zoonotic potential of new mutations or combinations of mutations and especially on the combination of traits, which could be impactful on the zoonotic potential. Bioinformatics tools can be used for the rapid identification of critical mutations on generated sequences. This will improve the understanding of their impact and help to identify novel mutations of concern and related traits.
- It is essential to draw up guidelines to ensure harmonisation in experimental characterisation studies, thereby enhancing the comparability of results and validating their reliability.

- The inventory of mutations enhancing virus zoonotic potential should be constantly updated through a periodic scanning of newly available studies.
- Harmonisation and standardisation of GISAID and other public sequence databases fields (e.g. host, species) are recommended, to allow systems interoperability and more efficient analysis.
- It is important to strengthen requirements for comprehensive metadata collection alongside viral sequences, including sampling date, geographic location, premise characteristics, exposure factors and host species. This information is critical for contextualising emerging mutations and their potential drivers.
- Further studies or different approaches are encouraged that may highlight additional trends and may lead to the production of further tools to support and improve the genomic surveillance of AI to strengthen the prevention of zoonotic risks.

5.1.2 | Conclusions from epidemiological analysis and seroprevalence studies in humans

Occurrence of human cases as an indicator of the zoonotic potential of different subtypes:

- A(H7N9), A(H5N1) and A(H9N2) subtypes have shown the highest occurrence in human cases, followed by A(H5N6) and A(H7N7), reflecting zoonotic potential. A(H5N1), A(H9N2) and A(H5N6) represent subtypes that have been reported to have caused human cases over decades and continue to be reported sporadically.
- Human cases caused by new and/or rarer subtypes are also sporadically detected (post-2021 data): A(H3N8), A(H5N2), A(H5N8), A(H10N3) and A(H10N5). Most of these zoonotic infections over the last decades were caused by viruses with internal genes of A(H9N2) virus origin, pointing at A(H9N2) viruses as a potential risk.
- A(H7N9) (the virus that has caused most reported human infections so far) has not been reported in humans since 2019, and A(H7N7) has not been reported in humans since 2013. A(H5N6) reported human cases have also declined in the past 2 years.
- A(H5N1) is the most widespread subtype geographically (in terms of reported human cases) with 24 countries having reported human cases so far. Out of a total of 46 AIV subtypes, all those that have been reported to have caused most human cases so far – i.e. A(H7N9), A(H9N2), A(H5N1), A(H5N6), A(H7N7) – but also subtypes with rarer occurrence in humans – e.g. A(H5N8), A(H10N8), A(H10N3), A(H3N8) – are among the subtypes that have also been identified through the mutation analysis as subtypes that tend to have a higher number of adaptive traits (3–5) (Figure 9).
- The recent increase in A(H5N1) human cases is mainly driven by cases in the USA and Cambodia. There are two clades that have been most commonly reported among human cases in the past 3 years: clade 2.3.4.4b in the USA and clade 2.3.2.1c (reassortant with clade 2.3.4.4b in most recent cases) in Cambodia.
- To date, confirmed human cases in EU/EEA have only been reported by the Netherlands in 2003 and Italy in 2013 and were of the A(H7N7) subtype; Spain has also reported two A(H5N1) cases in 2022 that were attributed to environmental contamination and were not considered true productive infections.
- There has been no sustained human-to-human transmission so far and only a few clusters of human cases have been reported (subtypes A(H5N1), A(H7N9) and A(H7N7)).

Subclinical infections, seroprevalence and immunity:

- It is expected that the level of immunity in the general population will be very low for all AI subtypes. Some pre-existing immunity from seasonal influenza viruses could potentially protect from severe disease, but further studies are needed to investigate whether cross-reactive NA antibodies confer protection against infection or modulate disease severity.
- Seroprevalence estimates were higher in exposed groups, especially for A(H9), but also for A(H5) and A(H7), indicating potential unrecognised asymptomatic infections in exposed individuals. A(H9N2) is one of the subtypes that have been associated with reported human cases in the past decades, but mainly in children, while data from seroprevalence studies show a higher risk of exposure in certain occupational groups as well.
- Most subclinical infections were detected in exposed individuals to AI in live bird markets (highlighting the risk from activities like butchering), backyard farms or commercial poultry farms that represent exposure groups at higher risk of infection. Recent data from dairy farms also showed a substantial number of A(H5N1) subclinical infections in workers in dairy farms in the US.
- There is a lack of seroprevalence studies in the EU/EEA; efforts should be made to conduct such studies using standardised methodologies to ensure comparability and reliability of results.

Severity and disease outcome after zoonotic infection:

- All AIV subtypes may have the potential to cause severe disease; however, certain subtypes like A(H9N2) have been linked to milder clinical presentations, while others have been causing more severe disease, like A(H7N9), A(H5N1) and A(H5N6).

- The decline in reported mortality rates for A(H5N1) may reflect a combination of factors, including different surveillance systems (increased detection of milder cases due to enhanced surveillance), exposure routes, viral characteristics and differences in the population groups exposed.
- The seroprevalence levels observed in exposed groups provide valuable context for interpreting case fatality rates, as they suggest that reported human cases may lead to an overestimation of fatality rates for A(H5) and A(H7) subtypes; this is because only severe cases are detected in many countries in the world (and reported to the WHO). The cases in the USA (linked to poultry and cattle H5N1 outbreaks in 2024) detected through a relatively sensitive surveillance systems, put in place to monitor occupationally exposed persons to infected animals, are showing that mild and asymptomatic cases may happen more frequently than estimated. Seroprevalence estimates support the assumption that a greater number of mild or asymptomatic cases are probably undetected.

Related recommendations have been incorporated in Section 5.2 on surveillance and Section 5.3 preventive measures below.

5.1.3 | Drivers and factors influencing the adaptation of AIV to mammals

- Drivers for the risk of AI adapting to mammals and potentially becoming zoonotic is multifaceted, involving viral genetics, human–animal interactions, ecological climatic factors, socio-economic factors and animal and public health preparedness.
- Genetic mutations and reassortment within the virus, combined with selective pressures like immune evasion and host-specific restriction factors, are primary mechanisms linked to the AIV characteristics that enhance the HPAIV's ability to infect and adapt to mammalian hosts (spillover), increasing zoonotic potential.
- External events and factors can also act as drivers for spillover transmission, thus enhancing zoonotic risk. Among these, (1) all human activities leading to increased direct and indirect contact among animals of different species, both wild and domestic, e.g. mixed farming practices, low biosecurity, farming close to wild bird-rich areas (wetlands), (2) wildlife ecology (e.g. predation behaviour) and (3) environmental and climatic changes impacting on animal demography (e.g. bird migration patterns, animal aggregation), significantly increase the risk of HPAI transmission across species, heightening the potential for the virus to adapt to new hosts, including humans.

Recommendation.

- Among long-term strategies to reduce the risk of spillover events, the structure of the livestock breeding system should be properly planned: the reduction of the density of commercial farms of highly susceptible species (both poultry and farmed mammals) primarily, but not only, in AI risk areas close to wetlands (high density of waterfowl) should be considered.
- Similarly, the reorganisation of certain animal production systems with low biosecurity in such areas, and/or livestock species often moved along the production chain (e.g. cattle movement in the USA) should be considered.

5.2 | Surveillance to address zoonotic risk of avian influenza

General recommendations:

- Surveillance of AIVs in both wild and domesticated animals and in humans to early detect spillover events and the possible emergence of mammalian-adaptive markers is essential to contain the spread of zoonotic AIVs.
- As the acquisition of mutations and phenotypic traits linked to increased zoonotic potential (covered in this Scientific Opinion) is a gradual process, effective surveillance systems and preventive measures on the animal and human health sides should be maintained over time.
- AI surveillance systems, both in animal and human health, should cover a wide range of AI subtypes, including those with a high number of traits/mutations associated with mammalian adaptation identified by the genetic analysis (see above in Section 5.1.1), especially focusing on areas with ongoing AI outbreaks in animals and population groups at higher risk of exposure.
- Close monitoring of A(H5Nx) viruses that are currently circulating in the EU is needed, considering their extensive circulation in birds and occasional spillover events in mammals, which provide opportunities for evolution.
- In addition to the list of mutations described in this document, it should be noted that other relevant mutations that may be to date less well studied need to be monitored, especially when they emerge in mammals and/or cause human infections. Vigilance about other mutations not described in this document (e.g. deletions, insertions, truncations, reassortments) is needed.
- LPAIVs that demonstrate the ability to infect humans should be monitored in any exposed susceptible animals and control measures aimed at reducing their spread should be implemented.

- Integration of virological, epidemiological, clinical and human seroprevalence data is needed for a comprehensive analysis that can inform joint veterinary/PH risk assessments for AIVs on their zoonotic and pandemic potential and guiding preventive and control measures.
- Countries should ensure timely reporting of human cases of AI infection and animal outbreaks, perform genomic surveillance and share sequencing data and associated metadata in international databases to allow monitoring of virus adaptation and zoonotic risk and for rapid international response coordination.
- Genomic surveillance should be performed on the whole genome. This was underlined by the fact that the mutations, identified in the genetic analysis and associated with different traits relevant to zoonotic evolution, are located in nine AI viral proteins.

5.2.1 | Animal health surveillance

The following scenarios are highlighted for AI surveillance in animal mammal species for detecting zoonotic and/or mammal-adapted AIV strains:

- Mammals with known exposure to AIV-infected birds or mammals may carry AIV mutations for mammal adaptation or zoonotic mutations.
  - *Recommendation*: Surveillance of AI in primarily[29] sick or found dead mammals with clear epidemiological connections to infected wild birds, poultry or mammals is recommended. The main target species under this scenario are companion animals, ruminants, pigs, rodents and fur animals (e.g. mink).
- Mammals found dead in areas and periods where and when AIV-infected birds or mammals have been recently reported may carry a virus with mutations for mammal adaptation or zoonotic mutations.
  - *Recommendation*: Surveillance of AI in mammals found dead during high-risk periods in areas of AIV circulation is recommended, even without direct epidemiological connection with infected wild birds, poultry or mammals. The most likely surveillance scenarios are wild mammals (in particular carnivores) and fur animals (e.g. mink) found dead, which can already be part of ongoing disease monitoring efforts and where AI surveillance could be added.
- Mammals with unexplained clinical signs in areas and periods where or when infected birds and mammals have been recently reported could also host AIV with mutations for mammal adaptation or zoonotic mutations.
  - *Recommendation*: Surveillance of AI in mammals with unexplained clinical signs during high-risk periods in areas of AIV circulation is recommended. This includes all categories of species (e.g. wild mammals, especially at rescue centres, rodents, companion animals, ruminants, fur animals and other domestic mammals).
- Sampling strategy depends on the population size and diversity within the epidemiological unit. AI outbreaks involving a large number of sick or found dead animals clustered in time and space (e.g. as observed in marine mammals) are probably due to a single strain (single introduction) rather than multiple different strains. Similarly, in farmed mammals, following a single introduction a single AIV variant is more likely to circulate within a farm rather than multiple variants.

  Recommendations

  - Sampling at least 10–15 sick or found dead individuals (clustering in time and space) (one sample per animal, if suitable for sequencing) is recommended per outbreak (close in time and space) and WGS should be performed on all positive ones (provided suitable), to allow detection of the molecular features of the predominant variant and any mutations for mammal adaptation or zoonotic mutations.
  - More samples for testing and sequencing can be taken according to laboratory capacity.
  - Sample collection in animals found dead should include at least the CNS and respiratory tract samples (e.g. tissue or swabs), although other tissues such as the lung, liver and spleen may also be considered to improve sensitivity. Sample collection in clinically diseased animals should be targeted at the organs causing the clinical signs.
  - In cases in which there are few target animals, e.g. infected companion animals in the households, all positive cases should be sequenced with WGS to determine the genetic sequence of AIV.
  - If multiple species within the epidemiological unit are affected, the selection of samples for WGS should cover all the species.
  - On dairy farms, sampling and WGS of approximately up to 10–15 individuals (one sample per individual) and bulk milk per epidemiological unit (herd) is recommended to allow detection of the molecular features of the predominant

[29] In the absence of dead or sick animals, sampling asymptomatic animals can also be considered based on decisions by local authorities.

variant. In surveillance activities, including bulk milk tests only, it should be highlighted that milk from animals suffering from mastitis should not enter the milk tank and that any dilution effect might affect sensitivity.

- Monitoring AIV strain evolution is necessary to provide insights into virus persistence, mutation and adaptation.
  - *Recommendation*: Repeated sampling and sequencing over time of the same affected epidemiological units (in case culling is not applied) to monitor strain evolution is recommended.
- Wildlife rescue centres receiving live, sick or asymptomatic wild animals, provide opportunities for AI surveillance, especially during high-risk periods of AI circulation.
  - *Recommendation*: Regular screening for species frequently infected with AI and animals showing unexplained clinical signs during high-risk periods or in high-risk areas is recommended through WGS.

5.2.2 | Public health surveillance

Recommendations.

- Active or passive surveillance and targeted testing of symptomatic exposed (e.g. occupationally) persons for early identification of zoonotic events should be performed. Testing of asymptomatic exposed people can be considered according to the level of exposure.
- Through routine influenza surveillance ideally all sentinel influenza-positive specimens from both primary and secondary care sources should be typed and subtyped.
- To identify sporadic severe human infections with the AI virus, enhanced surveillance should be considered in hospitals in the context of ongoing AI outbreaks in animals. Testing for influenza should be done according to clinical decisions and further typing and subtyping with a risk-based approach.
- While sustained human-to-human transmission has not been reported, vigilance is essential in detecting clusters or signs of limited transmission, particularly in high-exposure settings or during seasonal influenza peaks, when reassortment risk is higher.
- Any suspected AI sample (e.g. samples with inconclusive results for seasonal influenza) should be sent to the national reference laboratory for further subtyping and sequenced if found positive for AIV.
- Raising awareness to HCWs and clinicians about the epidemiological situation, symptoms of AIV infection and the importance of collecting data on potential sources of exposure/infection should be foreseen.
- Metadata associated with cases – symptoms, age, sex and potential sources of exposure/infection – should be systematically collected, as this information is not automatically collected in all cases (ECDC, online). Linking this information with sequences is crucial to improving understanding and mitigating infection risks.
- Timely notifications from all MSs of AIV infections in humans, as well as AI outbreaks in animals, are central to pandemic preparedness and prevention.
- MS are encouraged to comply with EU and international regulations for reporting AIV infections.
- Coordination between veterinary and public health sectors and cross-border collaboration and data sharing are important pillars for an effective response.

5.2.3 | Environmental surveillance including wastewater monitoring

- Environmental surveillance, such as surface water sampling, might be a complementary tool in monitoring AI by detecting and monitoring the virus in various natural settings, such as bird habitats and wetlands, adding insights into the AIV genetic evolution and diversity (if samples are suitable for WGS).
- Among environmental surveillance, wastewater monitoring is a cost-effective, non-invasive approach and could support providing indirect and broad insights into AIV circulation dynamics within a human community.

Recommendations

- Wastewater monitoring should be considered only as a complementary tool to traditional AIV surveillance methods as it cannot directly indicate the source of the virus (whether animal or human) or often the subtype.
- Studies about the effectiveness of wastewater monitoring for providing insights on AIV circulation in animal populations are still limited. Further research is needed to validate and determine the sensitivity of wastewater monitoring systems in detecting AIV subtypes, including how much AIV is needed to result in a positive detection and how this might vary with population size.

Further recommendations on OH outbreak investigations and management after the detection of AI virus in wastewater or environmental samples are provided in the recently published ECDC/EFSA technical report (ECDC and EFSA, 2025).

5.3 | Preparedness, control and prevention measures to reduce the risk of zoonotic avian influenza

The conclusions and related recommendations about prevention, protection and preparedness measures to reduce the risk of zoonotic AI under the One Health approach are listed below:

Measures at farm level and occupational settings:

- Implementation, compliance and maintenance of biosafety/biosecurity at the farm level is important to reduce the risk of HPAI introduction into a farm and thus the zoonotic risk of AI.

  Related recommendations

  - It is recommended that biosecurity measures tailored to each farm's unique topology and location are implemented and regularly checked.
  - Regular training for farm personnel, along with audits, is recommended to ensure compliance with biosecurity protocols.
  - Reporting to the animal health authorities of dead wild birds found near farms is recommended to increase alert levels.

- Besides biosecurity, the location of farms of susceptible species and the structure of breeding systems are important factors to prevent AI introduction and spread, which are favoured by a high density of commercial poultry farms, high number/density of highly susceptible mammal species (e.g. fur animals such as farmed mink), outdoor or in semi-open breeding systems and location in areas close to wetlands (high density of waterfowl).
- On HPAI-infected mammal farms, controlling HPAI spread within the farm and avoiding between-farm spread is crucial.

  Related recommendations

  - Infected mammals should be isolated or culled, and carcasses, as well as contaminated products, like manure and milk, must be properly disposed of.
  - Quarantine and movement restrictions on live animals from infected farms should remain in place until surveillance confirms the absence of the virus.
  - Contact tracing is essential to identify at-risk farms and the possible origin of infection.
  - If an AI outbreak in mammals occurs it is recommended to promptly apply control measures, to contain the spread of (mammal-adapted) virus at the smallest spatial and temporal scale.

- The recent infection of mammalian species previously not known to have been affected highlights the importance of having robust contingency plans for managing outbreaks.

  Related recommendations

  - Dedicated contingency plans related to farmed mammals must clearly set out the roles, actions and timelines for all stakeholders involved.
  - Sufficient capacity for surveillance, laboratory diagnostics, culling and response activities must be ensured.

- Vaccination of animals against AI can reduce virus circulation, protect PH and safeguard the poultry industry.

  Related recommendations

  - For vaccination to be effective, it must be properly integrated and implemented together with other preventive measures, including enhanced biosecurity and genomic as well as antigenic surveillance.
  - The circulation of AIV in poultry and the increased number of newly infected animal species increases the risk of human exposure and thus zoonotic risk.

  Related recommendations

  - Following an outbreak in animals, it is essential to inform and consult the national OSH authorities. Appropriate enhanced technical, organisational and personal workplace measures should be implemented by employers following a revision of the workplace risk assessment, including for operations such as culling and waste disposal.

- Health agencies must be prepared to respond to confirmed human cases in settings experiencing outbreaks in animals (having preparedness and response plans ready to be deployed at the local level, availability of resources).
- Active or passive follow-up of persons exposed to infected animals should be conducted for 10–14 days after the last exposure and testing of those with symptoms. Testing of asymptomatic exposed persons can be considered according to the level of exposure.
- Self-isolation of confirmed human cases, contact tracing and follow-up of exposed persons with confirmed human cases are recommended according to the ECDC published guidance.
- Seasonal influenza vaccination and PEP and treatment with antivirals should be provided according to national recommendations.
- Potential vaccination with the H5 vaccine of persons occupationally, or otherwise, routinely exposed to infected animals should be seen at this stage as an optional complementary measure based on national recommendations. Currently, there is insufficient evidence to recommend zoonotic influenza vaccination in all EU/EEA countries.
- Awareness of the zoonotic risk of AI should be raised and prevention at workplaces enhanced in collaboration with OSH authorities and the stakeholders from the sectors at risk, the agricultural community (e.g. farmers, agricultural workers, animal transporters, slaughterhouse personnel and veterinarians), health-care, dairy industry and food production, wildlife (managers, researchers, hunters), shelter personnel, pet keepers, etc.
- It is important to conduct regular OH simulation exercises including all the above-mentioned authorities and stakeholders to test and refine preparedness for AI outbreaks in animals (both in birds and in mammals) and to detect potential human cases.
- MS should invest in One Health approaches that consider both human and animal health and foster collaboration between PH, veterinary and environmental sectors for comprehensive AI risk management.

Measures for the general public and in the household:

- Clear communication with the general public is crucial for managing AI risks.

  Related recommendations

  - Information on AI risks must be accessible and accurate, emphasising good hygiene practices, such as avoiding contact with potentially infected animals and maintaining separation between poultry and living spaces.
  - Consumption of raw unpasteurised milk and raw milk-derived products produced in areas where AI outbreaks in dairy cattle farms are reported, should be avoided.
  - Prompt reporting of found sick or dead wild animals to authorities should be encouraged according to national recommendations.
  - Companion animals that roam outdoors are at increased risk of exposure to AI.

  Related recommendations

  - Owners should prevent their companion animals from coming into contact with dead wild birds, and signs of illness (respiratory, neurological) in companion animals, especially during high-risk periods and areas, should be reported to veterinarians.
  - In case of HPAI outbreaks on nearby farms, companion animals should be prevented from interacting with infected farm animals to avoid cross-species transmission.
  - In areas where AI outbreaks are reported in dairy cattle farms, feeding companion animals, especially cats, unpasteurised milk should be avoided, in addition to feeding them uncooked poultry or game bird products from areas experiencing outbreaks in birds.

- To date, there has been no evidence of transmission of AI virus to humans via dairy products and food in general. However, there is still uncertainty around these aspects. Furthermore, in the EU, animal products containing live viruses are unlikely to enter the food chain and industrial pasteurisation and recommended cooking practices play a significant role/are effective in inactivating AIV in milk, meat, eggs and their products.
- Backyard farms could represent a route of exposure for humans to AI (as do commercial farms), especially in areas affected by AI outbreaks.

  Related recommendations

  - In AI high-risk areas or periods, individuals within backyard poultry should follow strict hygiene rules, sick animals should be handled with appropriate protective measures, and any animal deaths or suspected disease should be reported to authorities for testing.

Measures for the wildlife:

- Managing AI outbreaks in wild mammals requires a coordinated approach between the involved stakeholders for both monitoring and control, in the natural environment, as well as for wild animal species kept in captivity such as in zoos, farms and wildlife rescue centres.
- Cornerstones to prevent AI in wild mammals include:
  - in free-ranging wildlife: biosecurity in wildlife handling, carcass removal and proper management of aspects that may increase density or interface between birds and mammals (e.g. artificial water bodies, habitat fragmentation, waste disposal sites);
  - for wild animals kept in captivity: biosecurity in handling and feeding, quarantine, species separation between birds and mammals and avoiding the location of premises in proximity to wild birds gathering sites.
- Among control measures against AI in wild mammals:
  - in free-ranging wildlife: surveillance in and beyond the outbreak area, reporting of found sick and dead animals, identification and compartmentalisation of outbreak area, biosecurity and use of PPE;
  - in wild animals kept in captivity: reporting of sick and dead animals, surveillance, biosecurity, cleaning and disinfection of premises, equipment and vehicles, waste removal, fomites and attractants, use of PPE; culling infected animals may be necessary, although isolation should be prioritised for species of conservation value.
- Preparedness measures for AI in wild mammals should focus on:
  - strengthening stakeholders' network and collaboration, both for free-ranging wildlife (hunters, managers, veterinary and forestry authorities) and wild animals kept in captivity (all personnel at farms, zoos, rescue centres, agriculture and veterinary authorities);
  - ad hoc legislation about wildlife management;
  - data collection, analysis and predictive models on wildlife distribution, abundance (both birds and mammals), spillover risk and land use and good communication of the results to relevant stakeholders.

ABBREVIATIONS

AI	Avian influenza
AIM	Avian influenza vaccine matching
AIV	Avian influenza virus
CNS	Central nervous system
CVV	Candidate vaccine virus
EA	Elbers ARW
ECDC	European Centre for Disease Prevention and Control
EIS	Event Information Site
EWRS	Early Warning Reporting System
GIP	Global Influenza Programme
HA	Haemagglutinin
HCW	Health-care workers
HERA	Health Emergency Preparedness and Response Authority
HPAI	Highly pathogenic avian influenza
HPAIV	Highly pathogenic avian influenza virus
IAV	Influenza A viruses
IHR	International Health Regulations
IRAT	Influenza Risk Assessment Tool
IVPI	Intravenous pathogenicity index
JRC	Joint Research Centre
LPAI	Low pathogenic avian influenza
LPAIV	Low pathogenic avian influenza virus
MS	Member States
NAAT	Nucleic acid amplification tests
NIH	National Institutes of Health
NP	Nucleoprotein
OSH	Occupational safety and health
PA	Polymerase acidic
PEP	Post-exposure prophylaxis
PH	Public health

PPE	Personal protective equipment
RBD	Receptor-binding domain
TIPRA	Tool for Influenza Pandemic Risk Assessment
URT	Upper respiratory tract
USDA	United States Department of Agriculture
WGS	Whole genome sequencing
WHO	World Health Organization
WOAH	World Organisation for Animal Health
WWM	Wastewater monitoring

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REFERENCES

Abdelwhab, E. M., & Mettenleiter, T. C. (2023). Zoonotic animal influenza virus and potential mixing vessel hosts. *Viruses*, *15*, 980. https://doi.org/10.3390/v15040980

Agüero, M., Monne, I., Sánchez, A., Zecchin, B., Fusaro, A., Ruano, M. J., Del Valle Arrojo, M., Fernández-Antonio, R., Souto, A. M., Tordable, P., Cañás, J., Bonfante, F., Giussani, E., Terregino, C., & Orejas, J. J. (2023). Highly pathogenic avian influenza A(H5N1) virus infection in farmed minks, Spain, October 2022. *Eurosurveillance*, *28*, 2300001. https://doi.org/10.2807/1560-7917.ES.2023.28.3.2300001

Aznar, E., Casas, I., González Praetorius, A., Ruano Ramos, M. J., Pozo, F., Sierra Moros, M. J., García Rivera, M. V., Sánchez Sánchez, A., García Villacieros, E., Saravia, G., Iglesias-Caballero, M., Román Marcos, E., & García San Miguel, L. (2023). Influenza A(H5N1) detection in two asymptomatic poultry farm workers in Spain, September to October 2022: Suspected environmental contamination. *Eurosurveillance*, *28*, 2300107. https://doi.org/10.2807/1560–7917.ES.2023.28.8.2300107

Banks, J., Speidel, E., & Alexander, D. J. (1998). Characterisation of an avian influenza a virus isolated from a human – Is an intermediate host necessary for the emergence of pandemic influenza viruses? *Archives of Virology*, *143*, 781–787. https://doi.org/10.1007/s007050050329

BAuA (Bundesanstalt für Arbeitsschutz und Arbeitsmedizin). (2007a). Beschluss 608: Empfehlung spezieller Maßnahmen zum Schutz der Beschäftigten vor Infektionen durch hochpathogene aviäre Influenzaviren (Klassische Geflügelpest, Vogelgrippe). Published in the Gemeinsames Ministerialblatt (GMBl). *Bundesanstalt für Arbeitsschutz und Arbeitsmedizin*, *19*, 403–407. https://www.baua.de/DE/Angebote/Regelwerk/TRBA/Beschluss-608

BAuA (Bundesanstalt für Arbeitsschutz und Arbeitsmedizin). (2007b). TRBA 230: protective measures for activities involving biological agents in agriculture and forestry and comparable activities (Technical Rule for Biological Agents). Published in the gemeinsames Ministerialblatt (GMBl), 14 February 2008. (Bundesanstalt für Arbeitsschutz und Arbeitsmedizin). pp. 72–81. https://www.baua.de/EN/Service/Technical-rules/TRBA/pdf/TRBA-230.pdf?__blob=publicationFile&v=1

Bauer, L., Benavides, F. F. W., Veldhuis Kroeze, E. J. B., de Wit, E., & Van Riel, D. (2023). The neuropathogenesis of highly pathogenic avian influenza H5Nx viruses in mammalian species including humans. *Trends in Neurosciences*, *46*, 953–970. https://doi.org/10.1016/j.tins.2023.08.002

Belser, J. A., Bridges, C. B., Katz, J. M., & Tumpey, T. M. (2009). Past, present, and possible future human infection with influenza virus a subtype H7. *Emerging Infectious Diseases*, *15*, 859–865. https://doi.org/10.3201/eid1506.090072

Belser, J. A., Lash, R. R., Garg, S., Tumpey, T. M., & Maines, T. R. (2018). The eyes have it: Influenza virus infection beyond the respiratory tract. *The Lancet Infectious Diseases*, *18*, e220–e227. https://doi.org/10.1016/S1473–3099(18)30102–6

Borges, V., Pinheiro, M., Pechirra, P., Guiomar, R., & Gomes, J. P. (2018). INSaFLU: An automated open web-based bioinformatics suite "from-reads" for influenza whole-genome-sequencing-based surveillance. *Genome Medicine*, *10*, 46. https://doi.org/10.1186/s13073-018-0555-0

Bos, M. E. H., Te Beest, D. E., van Boven, M., van Beest Holle, M. R.-D. R., Meijer, A., Bosman, A., Mulder, Y. M., Koopmans, M. P. G., & Stegeman, A. (2010). High probability of avian influenza virus (H7N7) transmission from poultry to humans active in disease control on infected farms. *Journal of Infectious Diseases*, *201*, 1390–1396. https://doi.org/10.1086/651663

Briand, F.-X., Souchaud, F., Pierre, I., Beven, V., Hirchaud, E., Hérault, F., Planel, R., Rigaudeau, A., Bernard-Stoecklin, S., Van der Werf, S., Lina, B., Gerbier, G., Eterradossi, N., Schmitz, A., Niqueux, E., & Grasland, B. (2023). Highly pathogenic avian influenza a(H5N1) clade 2.3.4.4b virus in domestic cat, France, 2022. *Emerging Infectious Diseases*, *29*, 1696–1698. https://doi.org/10.3201/eid2908.230188

Bridges, C. B., Katz, J. M., Seto, W. H., Chan, P. K., Tsang, D., Ho, W., Mak, K. H., Lim, W., Tam, J. S., Clarke, M., Williams, S. G., Mounts, A. W., Bresee, J. S., Conn, L. A., Rowe, T., Hu-Primmer, J., Abernathy, R. A., Lu, X., Cox, N. J., & Fukuda, K. (2000). Risk of influenza a (H5N1) infection among health care workers exposed to patients with influenza a (H5N1), Hong Kong. *Journal of Infectious Diseases*, *181*, 344–348. https://doi.org/10.1086/315213

Brigleb, P. H., Sharp, B., Roubidoux, E. K., Meliopoulos, V., Tan, S., Livingston, B., Morris, D., Ripperger, T., Lazure, L., Balaraman, V., Thompson, A. C., Kleinhenz, K., Dimitrov, K., Thomas, P. G., & Schultz-Cherry, S. (2024). Immune history modifies disease severity to HPAI H5N1 clade 2.3.4.4b viral challenge. *bioRxiv*, 619695. https://doi.org/10.1101/2024.10.23.619695

Brookes, S. M., Mansfield, K. L., Reid, S. M., Coward, V., Warren, C., Seekings, J., Brough, T., Gray, D., Núñez, A., & Brown, I. H. (2022). Incursion of H5N8 high pathogenicity avian influenza virus (HPAIV) into gamebirds in England. *Epidemiology and Infection*, *150*, e51. https://doi.org/10.1017/S0950268821002740

Burke, S. A., & Trock, S. C. (2018). Use of influenza risk assessment tool for prepandemic preparedness. *Emerging Infectious Diseases*, *24*, 471–477. https://doi.org/10.3201/eid2403.171852

Caliendo, V., Mensink, M., Begeman, L., Embregts, C., de Vrijer, M., De Baerdemaeker, A., Scheuer, R., Vuong, O., Fouchier, R. A. M., & Kuiken, T. (2022). Highly pathogenic avian influenza virus (H5n8) outbreak in a wild bird rescue center, The Netherlands: Consequences and recommendations. *Journal of Zoo and Wildlife Medicine*, *53*, 41–49. https://doi.org/10.1638/2021-0083

Caserta, L. C., Frye, E. A., Butt, S. L., Laverack, M., Nooruzzaman, M., Covaleda, L. M., Thompson, A. C., Prarat Koscielny, M., Cronk, B., Johnson, A., Kleinhenz, K., Edwards, E. E., Gomez, G., Hitchener, G., Martins, M., Kapczynski, D. R., Suarez, D. L., Alexander Morris, E. R., Hensley, T., … Diel, D. G. (2024). From birds to mammals: Spillover of highly pathogenic avian influenza H5N1 virus to dairy cattle led to efficient intra- and interspecies transmission. *bioRxiv*, 595317. https://doi.org/10.1101/2024.05.22.595317

CDC (Centers for Disease Control and Prevention). (online-a). CDC A(H5N1) bird flu response update June 14, 2024. https://www.cdc.gov/bird-flu/spotlights/h5n1-response-06142024.html

CDC (Centers for Disease Control and Prevention). (online-b). Influenza risk assessment tool (IRAT). https://www.cdc.gov/pandemic-flu/php/national-strategy/influenza-risk-assessment-tool.html

CDC (Centers for Disease Control and Prevention). (online-c). Past examples of probable limited, non-sustained, person-to-person spread of avian influenza A viruses. https://www.cdc.gov/bird-flu/php/avian-flu-summary/h5n1-human-infections.html

CDC (Centers for Disease Control and Prevention). (online-d). Results of influenza risk assessment tool. https://www.cdc.gov/pandemic-flu/php/monitoring/irat-virus-summaries.html

Chan, P. K. S. (2002). Outbreak of avian influenza a (H5N1) virus infection in Hong Kong in 1997. *Clinical Infectious Diseases*, *34*(Suppl 2), S58–S64. https://doi.org/10.1086/338820

Chan, R. W. Y., Yuen, K. M., Yu, W. C. L., Ho, C. C. C., Nicholls, J. M., Peiris, J. S. M., & Chan, M. C. W. (2010). Influenza H5N1 and H1N1 virus replication and innate immune responses in bronchial epithelial cells are influenced by the state of differentiation. *PLoS One*, *5*, e8713. https://doi.org/10.1371/journal.pone.0008713

Chaudhry, M., Webby, R., Swayne, D., Rashid, H. B., DeBeauchamp, J., Killmaster, L., Criado, M. F., Lee, D.-H., Webb, A., Yousaf, S., Asif, M., Ain, Q. U., Khan, M., Ilyas Khan, M., Hasan, S., Yousaf, A., Mushtaque, A., Bokhari, S. F., & Hasni, M. S. (2020). Avian influenza at animal-human interface: One-health challenge in live poultry retail stalls of Chakwal, Pakistan. *Influenza and Other Respiratory Viruses*, *14*, 257–265. https://doi.org/10.1111/irv.12718

Chen, H., Smith, G. J., Li, K. S., Wang, J., Fan, X. H., Rayner, J. M., Vijaykrishna, D., Zhang, J. X., Zhang, L. J., Guo, C. T., Cheung, C. L., Xu, K. M., Duan, L., Huang, K., Qin, K., Leung, Y. H., Wu, W. L., Lu, H. R., Chen, Y., … Guan, Y. (2006). Establishment of multiple sublineages of H5N1 influenza virus in Asia: Implications for pandemic control. *Proceedings of the National Academy of Sciences of the United States of America*, *103*, 2845–2850. https://doi.org/10.1073/pnas.0511120103

Chen, X., Wang, W., Wang, Y., Lai, S., Yang, J., Cowling, B. J., Horby, P. W., Uyeki, T. M., & Yu, H. (2020). Serological evidence of human infections with highly pathogenic avian influenza a(H5N1) virus: A systematic review and meta-analysis. *BMC Medicine*, *18*, 377. https://doi.org/10.1186/s12916-020-01836-y

Coman, A., Maftei, D. N., Krueger, W. S., Heil, G. L., Chereches, R. M., Sirlincan, E., Bria, P., Dragnea, C., Kasler, I., Valentine, M. A., & Gray, G. C. (2014). A prospective study of Romanian agriculture workers for zoonotic influenza infections. *PLoS One*, *9*, e98248. https://doi.org/10.1371/journal.pone.0098248

Daulagala, P., Cheng, S. M. S., Chin, A., Luk, L. L. H., Leung, K., Wu, J. T., Poon, L. L. M., Peiris, M., & Yen, H.-L. (2024). Avian influenza a(H5N1) neuraminidase inhibition antibodies in healthy adults after exposure to influenza a(H1N1)pdm09. *Emerging Infectious Diseases*, *30*, 168–171. https://doi.org/10.3201/eid3001.230756

de Bruin, A. C. M., Funk, M., Spronken, M. I., Gultyaev, A. P., Fouchier, R. A. M., & Richard, M. (2022). Hemagglutinin subtype specificity and mechanisms of highly pathogenic avian influenza virus genesis. *Viruses*, *14*, 1566. https://doi.org/10.3390/v14071566

de Jong, M. D., Tran, T. T., Truong, H. K., Vo, M. H., Smith, G. J., Nguyen, V. C., Bach, V. C., Phan, T. Q., Do, Q. H., Guan, Y., Peiris, J. S., Tran, T. H., & Farrar, J. (2005). Oseltamivir resistance during treatment of influenza a (H5N1) infection. *New England Journal of Medicine*, *353*, 2667–2672. https://doi.org/10.1056/NEJMoa054512

De Marco, M. A., Delogu, M., Facchini, M., Di Trani, L., Boni, A., Cotti, C., Graziosi, G., Venturini, D., Regazzi, D., Ravaioli, V., Marzadori, F., Frasnelli, M., Castrucci, M. R., & Raffini, E. (2021). Serologic evidence of occupational exposure to avian influenza viruses at the wildfowl/poultry/human interface. *Microorganisms*, *9*, 2153. https://doi.org/10.3390/microorganisms9102153

Delanglez, F., Ampe, B., Watteyn, A., Van Damme, L. G. W., & Tuyttens, F. A. M. (2024). How do Flemish laying hen farmers and private bird keepers comply with and think about measures to control avian influenza? *Veterinary Sciences*, *11*, 475. https://doi.org/10.3390/vetsci11100475

Dewulf, J., & Immerseel, F. (2019). *Biosecurity in animal production and veterinary medicine: From principles to practice* (p. 523). CABI Digital Library. https://www.cabidigitallibrary.org/doi/book/10.1079/9781789245684.0000

Domańska-Blicharz, K., Świętoń, E., Świątalska, A., Monne, I., Fusaro, A., Tarasiuk, K., Wyrostek, K., Styś-Fijoł, N., Giza, A., Pietruk, M., Zecchin, B., Pastori, A., Adaszek, Ł., Pomorska-Mól, M., Tomczyk, G., Terregino, C., & Winiarczyk, S. (2023). Outbreak of highly pathogenic avian influenza a(H5N1) clade 2.3.4.4b virus in cats, Poland, June to July 2023. *Eurosurveillance*, *28*, 2300366. https://doi.org/10.2807/1560-7917.ES.2023.28.31.2300366

Drehoff, C. C., White, E. B., Frutos, A. M., Stringer, G., Burakoff, A., Comstock, N., Cronquist, A., Alden, N., Armistead, I., Kohnen, A., Ratnabalasuriar, R., Travanty, E. A., Matzinger, S. R., Rossheim, A., Wellbrock, A., Pagano, H. P., Wang, D., Singleton, J., Sutter, R. A., … and H5N1 Field Investigation Team. (2024). Cluster of influenza a(H5) cases associated with poultry exposure at two facilities – Colorado, July 2024. *MMWR*, *73*, 734–739. https://doi.org/10.15585/mmwr.mm7334a1

Driskell, E. A., Jones, C. A., Berghaus, R. D., Stallknecht, D. E., Howerth, E. W., & Tompkins, S. M. (2013). Domestic cats are susceptible to infection with low pathogenic avian influenza viruses from shorebirds. *Veterinary Pathology*, *50*, 39–45. https://doi.org/10.1177/0300985812452578

ECDC (European Centre for Disease Prevention and Control). (2022a). Operational considerations for respiratory virus surveillance in Europe. https://www.ecdc.europa.eu/sites/default/files/documents/Operational-considerations-respiratory-virus-surveillance-euro-2022.pdf

ECDC (European Centre for Disease Prevention and Control). (2022b). Testing and detection of zoonotic influenza virus infections in humans in the EU/EEA, and occupational safety and health measures for those exposed at work. https://www.ecdc.europa.eu/sites/default/files/documents/zoonotic-influenza-virus-infections-testing-detection_0.pdf

ECDC (European Centre for Disease Prevention and Control). (2023a). Investigation protocol of human cases of avian influenza virus infections in EU/EEA. https://www.ecdc.europa.eu/sites/default/files/documents/Investigation-protocol-human-exposures-cases-avian-influenza.pdf

ECDC (European Centre for Disease Prevention and Control). (2023b). Survey report on laboratory capacity for molecular diagnosis and characterisation of zoonotic influenza viruses in human specimens in EU/EEA and the western Balkans. https://www.ecdc.europa.eu/sites/default/files/documents/survey-report-lab%20capacity-zoonotic-influenza-december-2023.pdf

ECDC (European Centre for Disease Prevention and Control). (2024a). Enhanced influenza surveillance to detect avian influenza virus infections in the EU/EEA during the inter-seasonal period. https://www.ecdc.europa.eu/sites/default/files/documents/Enhanced%20test%20of%20severe%20avian%20influenza%20virus%20infections.pdf

ECDC (European Centre for Disease Prevention and Control). (2024b). Surveillance and targeted testing for the early detection of zoonotic influenza in humans during the winter period in the EU/EEA. https://www.ecdc.europa.eu/sites/default/files/documents/surveillance-testing-early-detection-zoonotic-flu.pdf

ECDC (European Centre for Disease Prevention and Control). (online). Respiratory virus surveillance and study protocols. https://www.ecdc.europa.eu/en/covid-19/surveillance/study-protocols

ECDC and EFSA (European Centre for Disease Prevention and Control and European Food Safety Authority), Hallmeier-Wacker, L., Enkirch, T., Melidou, A., Willgert, K., & Gervelmeyer, A. (2025). Coordinated One Health investigation and management of outbreaks in humans and animals caused by zoonotic avian influenza viruses - 9 December 2024, ISBN 978-92-9498-765-5; doi: 10.2900/4758784 Catalogue number: TQ-01-24-021-EN-N. https://doi. org/10.2903/j.efsa.2025.9183

EFSA (European Food Safety Authority), Aznar, I., Kohnle, L., Stoicescu, A., van Houtum, A., & Zancanaro, G. (2023). Annual report on surveillance for avian influenza in poultry and wild birds in member states of the European Union in 2022. *EFSA Journal*, *21*(12), 8480. https://doi.org/10.2903/j.efsa.2023.8480

EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), More, S., Bicout, D., Bøtner, A., Butterworth, A., Calistri, P., Depner, K., Edwards, S., Garin-Bastuji, B., Good, M., Gortázar Schmidt, C., Michel, V., Miranda, M. A., Nielsen, S. S., Raj, M., Sihvonen, L., Spoolder, H., Thulke, H.-H., Velarde, A., … Stegeman, J. A. (2017). Avian influenza. *EFSA Journal*, *15*(10), 4991. https://doi.org/10.2903/j.efsa.2017.4991

EFSA AHAW Panel (EFSA Panel on Animal Health and Animal Welfare) European Union reference laboratory for avian influenza, Nielsen, S. S., Álvarez, J., Bicout, D. J., Calistri, P., Canali, E., Drewe, J. A., Garin-Bastuji, B., Gonzales Rojas, J. L., Gortázar, C., Herskin, M., Michel, V., Chueca, M. A. M., Padalino, B., Roberts, H. C., Spoolder, H., Stahl, K., Velarde, A., Winckler, C., … Viltrop, A. (2023). Vaccination of poultry against highly pathogenic avian influenza – Part 1. Available vaccines and vaccination strategies. *EFSA Journal*, *21*(10), 8271. https://doi.org/10.2903/j.efsa.2023.8271

EFSA AHAW Panel (EFSA Panel on Animal Health and Animal Welfare) European Union reference laboratory for avian influenza, Nielsen, S. S., Álvarez, J., Bicout, D. J., Calistri, P., Canali, E., Drewe, J. A., Garin-Bastuji, B., Gortázar, C., Herskin, M. S., Michel, V., Miranda Chueca, M. Á., Padalino, B., Roberts, H. C., Spoolder, H., Stahl, K., Velarde, A., Viltrop, A., Winckler, C., … Gonzales Rojas, J. L. (2024). Vaccination of poultry against highly pathogenic avian influenza – Part 2. Surveillance and mitigation measures. *EFSA Journal*, *22*(4), 70. https://doi.org/10.2903/j.efsa.2024.8755

EFSA and ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control), Adlhoch, C., Alm, E., Enkirch, T., Lamb, F., Melidou, A., Willgert, K., Marangon, S., Monne, I., Stegeman, J. A., Delacourt, R., Baldinelli, F., & Broglia, A. (2024). Drivers for a pandemic due to avian influenza and options for one health mitigation measures. *EFSA Journal*, *22*(4), 8735. https://doi.org/10.2903/j.efsa.2024.8735

EFSA, ECDC, and EURLEU (European Food Safety Authority, European Centre for Disease Prevention and Control, and European Union Reference Laboratory for Avian Influenza), Adlhoch, C., Fusaro, A., Gonzales, J. L., Kuiken, T., Melidou, A., Mirinavičiūtė, G., Niqueux, É., Ståhl, K., Staubach, C., Terregino, C., Baldinelli, F., Broglia, A., & Kohnle, L. (2023). Avian influenza overview April–June 2023. *EFSA Journal*, *21*(7), 8191. https://doi.org/10.2903/j.efsa.2023.8191

EFSA, ECDC, and EURLEU (European Food Safety Authority, European Centre for Disease Prevention and Control, and European Union Reference Laboratory for Avian Influenza), Alexakis, L., Buczkowski, H., Ducatez, M., Fusaro, A., Gonzales, J. L., Kuiken, T., StåhlK, K., Staubach, C., Svartström, O., Terregino, C., Willgert, K., Delacourt, R., & Kohnle, L. (2024a). Scientific report: Avian influenza overview June–September 2024. *EFSA Journal*, *22*(10), 9057. https://doi.org/10.2903/j.efsa.2024.9057

EFSA, ECDC, and EURLEU (European Food Safety Authority, European Centre for Disease Prevention and Control, and European Union Reference Laboratory for Avian Influenza), Alexakis, L., Fusaro, A., Kuiken, T., Mirinavičiūtė, G., Ståhl, K., Staubach, C., Svartström, O., Terregino, C., Willgert, K., Delacourt, R., Goudjihounde, S. M., Tampach, S., & Kohnle, L. (2024b). Scientific report: Avian influenza overview March–June 2024. *EFSA Journal*, *22*(7), 8930. https://doi.org/10.2903/j.efsa.2024.8930

EFSA, ECDC, and EURLEU (European Food Safety Authority, European Centre for Disease Prevention and Control, and European Union Reference Laboratory for Avian Influenza), Fusaro, A., Gonzales, J., Kuiken, T., Mirinavičiūtė, G., Niqueux, É., Ståhl, K., Staubach, C., Svartström, O., Terregino, C., Willgert, K., Baldinelli, F., Delacourt, R., Georganas, A., & Kohnle, L. (2024c). Scientific report: Avian influenza overview December 2023–march 2024. *EFSA Journal*, *22*, 8754. https://doi.org/10.2903/j.efsa.2024.8754

Eisfeld, A. J., Biswas, A., Guan, L., Gu, C., Maemura, T., Trifkovic, S., Wang, T., Babujee, L., Dahn, R., Halfmann, P. J., Barnhardt, T., Neumann, G., Suzuki, Y., Thompson, A., Swinford, A. K., Dimitrov, K. M., Poulsen, K., & Kawaoka, Y. (2024). Pathogenicity and transmissibility of bovine H5N1 influenza virus. *Nature*, *633*(8029), 426–432. https://doi.org/10.1038/s41586-024-07766-6

El Masry, I., Delgado, A. H., Silva, G. O. D., Dhingra, M., & Lyons, N. A. (2024). Recommendations for the surveillance of influenza a(H5N1) in cattle – With broader application to other farmed mammals. FAO animal production and health guidelines, No. 37. FAO, Rome.

El-Shesheny, R., Franks, J., Kandeil, A., Badra, R., Turner, J., Seiler, P., Marathe, B. M., Jeevan, T., Kercher, L., Hu, M., Sim, Y. E., Hui, K. P. Y., Chan, M. C. W., Thompson, A. J., McKenzie, P., Govorkova, E. A., Russell, C. J., Vogel, P., Paulson, J. C., … Webby, R. J. (2024). Cross-species spill-over potential of the H9N2 bat influenza A virus. *Nature Communications*, *15*, 3449. https://doi.org/10.1038/s41467-024-47635-4

EMA (European Medicines Agency). (online). Vaccines for pandemic influenza. https://www.ema.europa.eu/en/human-regulatory-overview/public-health-threats/pandemic-influenza/vaccines-pandemic-influenza

ENETwild consortium, Occhibove, F., Knauf, S., Sauter, L. C., Staubach, C., Allendorf, V., Anton, A., Barron, S., Bergmann, H., Bröjer, C., Buzan, E., Cerny, J., Denzin, N., Gethöffer, F., Globig, A., Gethmann, J., Gonzálvez, M., García-Bocanegra, I., Harder, T., … Ferroglio, E. (2024a). The role of mammals in avian influenza: A review. *EFSA Supporting Publications*, 8692. https://doi.org/10.2903/sp.efsa.2024.EN-8692

ENETwild consortium, Vada, R., Caliendo, V., Gonzálvez, M., Buzan, E., Bussi, M., Caliendo, V., Palumbo, L., García-Bocanegra, I., Zanet, S., Ferroglio, E., & Rijks, J. (2024b). Highly pathogenic avian influenza in wild mammals: Critical appraisal of spill-over events and of strategies for prevention, surveillance and preparedness. https://doi.org/10.5281/zenodo.14002535

EU-OSHA (European Agency for Occupational Safety and Health). (2019). Exposure to biological agents and related health problems in animal-related occupations. https://osha.europa.eu/sites/default/files/Biological_agents_in_animal_related_occupations.pdf

EU-OSHA (European Agency for Occupational Safety and Health). (online-a). Directive 89/391/EEC - OSH "Framework Directive". https://osha.europa.eu/en/legislation/directives/the-osh-framework-directive/1

EU-OSHA (European Agency for Occupational Safety and Health). (online-b). Directive 2000/54/EC – Biological Agents at Work. https://osha.europa.eu/es/legislation/directives/exposure-to-biological-agents/77

EU-Wastewater Observatory for Public Health. (online). GLOWACON. https://wastewater-observatory.jrc.ec.europa.eu/#/content/glowacon-new

EU-WISH (EU-Wastewater Integrated Surveillance for Public Health). (online). EU-Wastewater Integrated Surveillance for Public Health. https://www.eu-wish.eu/

Everest, H., Billington, E., Daines, R., Burman, A., & Iqbal, M. (2021). The emergence and zoonotic transmission of H10Nx avian influenza virus infections. *MBio*, *12*, e0178521. https://doi.org/10.1128/mBio.01785–21

FAO, WHO, and WOAH (Food and Agriculture Organization of the United Nations, World Health Organization, and World Organisation for Animal Health). (2024a). Joint FAO/WHO/WOAH preliminary assessment of recent influenza A(H5N1) viruses. https://cdn.who.int/media/docs/default-source/global-influenza-programme/2024_04_23_fao-woah-who_h5n1_assessment.pdf

FAO, WHO, and WOAH (Food and Agriculture Organization of the United Nations, World Health Organization, and World Organisation for Animal Health). (2024b). Updated joint FAO/WHO/WOAH assessment of recent influenza A(H5N1) virus events in animals and people.

Fasina, F. O., Ifende, V. I., & Ajibade, A. A. (2010). Avian influenza a(H5N1) in humans: Lessons from Egypt. *Eurosurveillance*, *15*, 19473. https://doi.org/10.2807/ese.15.04.19473-en

Floyd, T., Banyard, A. C., Lean, F. Z. X., Byrne, A. M. P., Fullick, E., Whittard, E., Mollett, B. C., Bexton, S., Swinson, V., Macrelli, M., Lewis, N. S., Reid, S. M., Núñez, A., Duff, J. P., Hansen, R., & Brown, I. H. (2021). Encephalitis and death in wild mammals at a rehabilitation center after infection with highly pathogenic avian influenza a(H5N8) virus, United Kingdom. *Emerging Infectious Diseases*, *27*, 2856–2863. https://doi.org/10.3201/eid2711.211225

Fouchier, R. A. M., Schneeberger, P. M., Rozendaal, F. W., Broekman, J. M., Kemink, S. A. G., Munster, V., Kuiken, T., Rimmelzwaan, G. F., Schutten, M., van Doornum, G. J. J., Koch, G., Bosman, A., Koopmans, M., & Osterhaus, A. D. M. E. (2004). Avian influenza a virus (H7N7) associated with human conjunctivitis and a fatal case of acute respiratory distress syndrome. *Proceedings of the National Academy of Sciences of the United States of America*, *101*, 1356–1361. https://doi.org/10.1073/pnas.0308352100

Frymus, T., Belák, S., Egberink, H., Hofmann-Lehmann, R., Marsilio, F., Addie, D. D., Boucraut-Baralon, C., Hartmann, K., Lloret, A., Lutz, H., Pennisi, M. G., Thiry, E., Truyen, U., Tasker, S., Möstl, K., & Hosie, M. J. (2021). Influenza virus infections in cats. *Viruses*, *13*, 1435. https://doi.org/10.3390/v13081435

Garretson, T. A., Liu, J., Li, S. H., Scher, G., Santos, J. J. S., Hogan, G., Vieira, M. C., Furey, C., Atkinson, R. K., Ye, N., Ort, J., Kim, K., Hernandez, K. A., Eilola, T., Schultz, D. C., Cherry, S., Cobey, S., & Hensley, S. E. (2024). Immune history shapes human antibody responses to H5N1 influenza viruses. *medRxiv*, 24316514. https://doi.org/10.1101/2024.10.31.24316514

Gary, F., Clauss, M., Bonbon, E., & Myers, L. (2021). Good emergency management practice: The essentials – A guide to preparing for animal health emergencies. 3rd edition. FAO Animal Production and Health Manual No. 25. FAO, Rome.

GenoFU. (online). Influenza data pipeline to automate genotyping assignment. GitHub. https://github.com/USDA-VS/GenoFLU

Germeraad, E. A., Elbers, A. R. W., de Bruijn, N. D., Heutink, R., van Voorst, W., Hakze-van der Honing, R., Bergervoet, S. A., Engelsma, M. Y., van der Poel, W. H. M., & Beerens, N. (2020). Detection of low pathogenic avian influenza virus subtype H10N7 in poultry and environmental water samples during a clinical outbreak in commercial free-range layers, Netherlands 2017. *Frontiers in Veterinary Science*, *7*, 237. https://doi.org/10.3389/fvets.2020.00237

Gomaa, M., Moatasim, Y., El Taweel, A., Mahmoud, S. H., El Rifay, A. S., Kandeil, A., McKenzie, P. P., Webby, R. J., El-Shesheny, R., Ali, M. A., & Kayali, G. (2023). We are underestimating, again, the true burden of H5N1 in humans. *BMJ Global Health*, *8*, e013146. https://doi.org/10.1136/bmjgh-2023-013146

Gostic, K. M., Ambrose, M., Worobey, M., & Lloyd-Smith, J. O. (2016). Potent protection against H5N1 and H7N9 influenza via childhood hemagglutinin imprinting. *Science*, *354*(6313), 722–726. https://doi.org/10.1126/science.aag1322

Götz, V., Magar, L., Dornfeld, D., Giese, S., Pohlmann, A., Höper, D., Kong, B.-W., Jans, D. A., Beer, M., Haller, O., & Schwemmle, M. (2016). Corrigendum: Influenza a viruses escape from MxA restriction at the expense of efficient nuclear vRNP import. *Scientific Reports*, *6*, 25428. https://doi.org/10.1038/srep25428

Gourlay, P., Decors, A., Moinet, M., Lambert, O., Lawson, B., Beaudeau, F., & Assié, S. (2014). The potential capacity of French wildlife rescue centres for wild bird disease surveillance. *European Journal of Wildlife Research*, *60*, 865–873. https://doi.org/10.1007/s10344-014-0853-9

Gu, C., Maemura, T., Guan, L., Eisfeld, A. J., Biswas, A., Kiso, M., Uraki, R., Ito, M., Trifkovic, S., Wang, T., Babujee, L., Presler, R., Dahn, J., R, Suzuki, Y., Halfmann, P. J., Yamayoshi, S., Neumann, G., & Kawaoka, Y. (2024). A human isolate of bovine H5N1 is transmissible and lethal in animal models. *Nature*. https://doi.org/10.1038/s41586-024-08254-7

Guan, L., Eisfeld, A. J., Pattinson, D., Gu, C., Biswas, A., Maemura, T., Trifkovic, S., Babujee, L., Presler, R., Jr., Dahn, R., Halfmann, P. J., Barnhardt, T., Neumann, G., Thompson, A., Swinford, A. K., Dimitrov, K. M., Poulsen, K., & Kawaoka, Y. (2024). Cow's milk containing avian influenza a(H5N1) virus – Heat inactivation and infectivity in mice. *New England Journal of Medicine*, *391*, 87–90. https://doi.org/10.1056/NEJMc2405495

Hall, V., Cardona, C., Mendoza, K., Torchetti, M., Lantz, K., Bueno, I., & Franzen-Klein, D. (2024). Surveillance for highly pathogenic avian influenza a (H5N1) in a raptor rehabilitation center—2022. *PLoS One*, *19*, e0299330. https://doi.org/10.1371/journal.pone.0299330

Halwe, N. J., Hamberger, L., Sehl-Ewert, J., Mache, C., Schön, J., Ulrich, L., Calvelage, S., Tönnies, M., Fuchs, J., Bandawane, P., Loganathan, M., Abbad, A., Carreño, J. M., Bermúdez-González, M. C., Simon, V., Kandeil, A., El-Shesheny, R., Ali, M. A., Kayali, G., … Beer, M. (2024). Bat-borne H9N2 influenza virus evades MxA restriction and exhibits efficient replication and transmission in ferrets. *Nature Communications*, *15*, 3450. https://doi.org/10.1038/s41467-024-47455-6

Hinshaw, V. S., Webster, R. G., Easterday, B. C., & Bean, W. J., Jr. (1981). Replication of avian influenza a viruses in mammals. *Infection and Immunity*, *34*, 354–361. https://doi.org/10.1128/iai.34.2.354-361.1981

Hobbelen, P. H. F., Elbers, A. R. W., Werkman, M., Koch, G., Velkers, F. C., Stegeman, A., & Hagenaars, T. J. (2020). Estimating the introduction time of highly pathogenic avian influenza into poultry flocks. *Scientific Reports*, *10*, 12388. https://doi.org/10.1038/s41598-020-68623-w

Honein, M. A., Olsen, S. J., Jernigan, D. B., & Daskalakis, D. C. (2024). Challenges and opportunities for wastewater monitoring of influenza viruses during the multistate outbreak of highly pathogenic avian influenza a(H5N1) virus in dairy cattle and poultry. *American Journal of Public Health*, *114*, 1309–1312. https://doi.org/10.2105/AJPH.2024.307860

Horm, S. V., Tarantola, A., Rith, S., Ly, S., Gambaretti, J., Duong, V., Y, P., Sorn, S., Holl, D., Allal, L., Kalpravidh, W., Dussart, P., Horwood, P. F., & Buchy, P. (2016). Intense circulation of a/H5N1 and other avian influenza viruses in Cambodian live-bird markets with serological evidence of sub-clinical human infections. *Emerging Microbes & Infections*, *5*, e70. https://doi.org/10.1038/emi.2016.69

Hu, X., Saxena, A., Magstadt, D. R., Gauger, P. C., Burrough, E., Zhang, J., Siepker, C., Mainenti, M., Gorden, P. J., Plummer, P., & Li, G. (2024). Highly pathogenic avian influenza a (H5N1) clade 2.3.4.4b virus detected in dairy cattle. *bioRxiv*. https://doi.org/10.1101/2024.04.16.588916

Imai, M., Watanabe, T., Hatta, M., Das, S. C., Ozawa, M., Shinya, K., Zhong, G., Hanson, A., Katsura, H., Watanabe, S., Li, C., Kawakami, E., Yamada, S., Kiso, M., Suzuki, Y., Maher, E. A., Neumann, G., & Kawaoka, Y. (2012). Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets. *Nature*, *486*(7403), 420–428. https://doi.org/10.1038/nature10831

INRS (Institut National de Recherche et de Sécurité). (2017). Ventilation des postes d'accrochage en abattoir de volailles. https://www.inrs.fr/media.html?refINRS=ED%206279

Kareinen, L., Tammiranta, N., Kauppinen, A., Zecchin, B., Pastori, A., Monne, I., Terregino, C., Giussani, E., Kaarto, R., Karkamo, V., Lähteinen, T., Lounela, H., Kantala, T., Laamanen, I., Nokireki, T., London, L., Helve, O., Kääriäinen, S., Ikonen, N., … Gadd, T. (2024). Highly pathogenic avian influenza a(H5N1) virus infections on fur farms connected to mass mortalities of black-headed gulls, Finland, July to October 2023. *Eurosurveillance*, *29*, 2400063. https://doi.org/10.2807/1560-7917.ES.2024.29.25.2400063

Kelly, T. R., Pandit, P. S., Carion, N., Dombrowski, D. F., Rogers, K. H., McMillin, S. C., Clifford, D. L., Riberi, A., Ziccardi, M. H., Donnelly-Greenan, E. L., & Johnson, C. K. (2021). Early detection of wildlife morbidity and mortality through an event-based surveillance system. *Proceedings of the Biological Sciences*, *288*, 20210974. https://doi.org/10.1098/rspb.2021.0974

Khan, S. U., Anderson, B. D., Heil, G. L., Liang, S., & Gray, G. C. (2015). A systematic review and meta-analysis of the seroprevalence of influenza a(H9N2) infection among humans. *Journal of Infectious Diseases*, *212*, 562–569. https://doi.org/10.1093/infdis/jiv109

Klopfleisch, R., Wolf, P. U., Uhl, W., Gerst, S., Harder, T., Starick, E., Vahlenkamp, T. W., Mettenleiter, T. C., & Teifke, J. P. (2007). Distribution of lesions and antigen of highly pathogenic avian influenza virus a/swan/Germany/R65/06 (H5N1) in domestic cats after presumptive infection by wild birds. *Veterinary Pathology*, *44*, 261–268. https://doi.org/10.1354/vp.44-3-261

Korteweg, C., & Gu, J. (2008). Pathology, molecular biology, and pathogenesis of avian influenza a (H5N1) infection in humans. *American Journal of Pathology*, *172*, 1155–1170. https://doi.org/10.2353/ajpath.2008.070791

Kozlov, M. (2024). Huge amounts of bird-flu virus found in raw milk of infected cows. *Nature*. https://doi.org/10.1038/d41586–024–01624–1

Krog, J. S., Hansen, M. S., Holm, E., Hjulsager, C. K., Chriél, M., Pedersen, K., Andresen, L. O., Abildstrøm, M., Jensen, T. H., & Larsen, L. E. (2015). Influenza a(H10N7) virus in dead harbor seals, Denmark. *Emerging Infectious Diseases*, *21*, 684–687. https://doi.org/10.3201/eid2104.141484

Kuiken, T., Fouchier, R., Rimmelzwaan, G., Osterhaus, A., & Roeder, P. (2006). Feline friend or potential foe? *Nature*, *440*(7085), 741–742. https://doi.org/10.1038/440741a

Kwon, J.-H., Bertran, K., Lee, D.-H., Criado, M. F., Killmaster, L., Pantin-Jackwood, M. J., & Swayne, D. E. (2023). Diverse infectivity, transmissibility, and pathobiology of clade 2.3.4.4 H5Nx highly pathogenic avian influenza viruses in chickens. *Emerging Microbes & Infections*, *12*, 2218945. https://doi.org/10.1080/22221751.2023.2218945

Lang, G., Gagnon, A., & Geraci, J. R. (1981). Isolation of an influenza a virus from seals. *Archives of Virology*, *68*(3–4), 189–195. https://doi.org/10.1007/BF01314571

Le Sage, V., Campbell, A. J., Reed, D. S., Duprex, W. P., & Lakdawala, S. S. (2024). Influenza H5N1 and H1N1 viruses remain infectious in unpasteurized milk on milking machinery surfaces. *medRxiv*. https://doi.org/10.1101/2024.05.22.24307745

Le Sage, V., Werner, B. D., Merrbach, G. A., Petnuch, S. E., O'Connell, A. K., Simmons, H. C., McCarthy, K. R., Reed, D. S., Moncla, L. H., Bhavsar, D., Krammer, F., Crossland, N. A., McElroy, A. K., Duprex, W. P., & Lakdawala, S. S. (2024). Pre-existing H1N1 immunity reduces severe disease with bovine H5N1 influenza virus. *bioRxiv*. https://doi.org/10.1101/2024.10.23.619881

Lee, A. J., Carson, S., Reyne, M. I., Marshall, A., Moody, D., Allen, D. M., Allingham, P., Levickas, A., Fitzgerald, A., Bell, S. H., Lock, J., Coey, J. D., McSparron, C., Nejad, B. F., Troendle, E. P., Simpson, D. A., Courtney, D. G., Einarsson, G. G., McKenna, J. P., … Bamford, C. G. G. (2024). Wastewater monitoring of human and avian influenza a viruses in Northern Ireland: A genomic surveillance study. *Lancet Microbe*, *5*, 100933. https://doi.org/10.1016/S2666-5247(24)00175-7

Lee, L. Y.-H., Do Ha, L. A., Simmons, C., de Jong, M. D., Chau, N. V. V., Schumacher, R., Peng, Y. C., McMichael, A. J., Farrar, J. J., Smith, G. L., Townsend, A. R. M., Askonas, B. A., Rowland-Jones, S., & Dong, T. (2008). Memory T cells established by seasonal human influenza a infection cross-react with avian influenza a (H5N1) in healthy individuals. *Journal of Clinical Investigation*, *118*, 3478–3490. https://doi.org/10.1172/JCI32460

Li, Y.-T., Linster, M., Mendenhall, I. H., Su, Y. C. F., & Smith, G. J. D. (2019). Avian influenza viruses in humans: Lessons from past outbreaks. *British Medical Bulletin*, *132*, 81–95. https://doi.org/10.1093/bmb/ldz036

Lin, L., Wang, X., Chen, Z., Deng, T., Yan, Y., Dong, W., Huang, Y., & Zhou, J. (2023). TRIM21 restricts influenza a virus replication by ubiquitination-dependent degradation of M1. *PLoS Pathogens*, *19*, e1011472. https://doi.org/10.1371/journal.ppat.1011472

Lindh, E., Lounela, H., Ikonen, N., Kantala, T., Savolainen-Kopra, C., Kauppinen, A., Österlund, P., Kareinen, L., Katz, A., Nokireki, T., Jalava, J., London, L., Pitkäpaasi, M., Vuolle, J., Punto-Luoma, A.-L., Kaarto, R., Voutilainen, L., Holopainen, R., Kalin-Mänttäri, L., … Salminen, M. (2023). Highly pathogenic avian influenza a(H5N1) virus infection on multiple fur farms in the south and Central Ostrobothnia regions of Finland, July 2023. *Eurosurveillance*, *28*, 2300400. https://doi.org/10.2807/1560–7917.ES.2023.28.31.2300400

Linster, M., van Boheemen, S., de Graaf, M., Schrauwen, E. J. A., Lexmond, P., Mänz, B., Bestebroer, T. M., Baumann, J., van Riel, D., Rimmelzwaan, G. F., Osterhaus, A. D. M. E., Matrosovich, M., Fouchier, R. A. M., & Herfst, S. (2014). Identification, characterization, and natural selection of mutations driving airborne transmission of a/H5N1 virus. *Cell*, *157*, 329–339. https://doi.org/10.1016/j.cell.2014.02.040

Liu, W. J., Xiao, H., Dai, L., Liu, D., Chen, J., Qi, X., Bi, Y., Shi, Y., Gao, G. F., & Liu, Y. (2021). Avian influenza a (H7N9) virus: From low pathogenic to highly pathogenic. *Frontiers of Medicine*, *15*, 507–527. https://doi.org/10.1007/s11684-020-0814-5

Louis, S., Mark-Carew, M., Biggerstaff, M., Yoder, J., Boehm, A. B., Wolfe, M. K., Flood, M., Peters, S., Stobierski, M. G., Coyle, J., Leslie, M. T., Sinner, M., Nims, D., Salinas, V., Lustri, L., Bojes, H., Shetty, V., Burnor, E., Rabe, A., … Honein, M. A. (2024). Wastewater surveillance for influenza a virus and H5 subtype concurrent with the highly pathogenic avian influenza a(H5N1) virus outbreak in cattle and poultry and associated human cases - United States, may 12-July 13, 2024. *MMWR*, *73*, 804–809. https://doi.org/10.15585/mmwr.mm7337a1

Ly, H. (2024). Highly pathogenic avian influenza H5N1 virus infections of dairy cattle and livestock handlers in The United States of America. *Virulence*, *15*, 2343931. https://doi.org/10.1080/21505594.2024.2343931

Lyoo, K. S., Na, W., Phan, L. V., Yoon, S. W., Yeom, M., Song, D., & Jeong, D. G. (2017). Experimental infection of clade 1.1.2 (H5N1), clade 2.3.2.1c (H5N1) and clade 2.3.4.4 (H5N6) highly pathogenic avian influenza viruses in dogs. *Transboundary and Emerging Diseases*, *64*, 1669–1675. https://doi.org/10.1111/tbed.12731

Ma, M.-J., Ma, G.-Y., Yang, X.-X., Chen, S.-H., Gray, G. C., Zhao, T., Bao, J., Zhou, J.-J., Qian, Y.-H., Lu, B., Ling, X., & Cao, W.-C. (2015). Avian influenza a(H7N9) virus antibodies in close contacts of infected persons, China, 2013–2014. *Emerging Infectious Diseases*, *21*, 709–711. https://doi.org/10.3201/eid2104.141442

Matrosovich, M., Tuzikov, A., Bovin, N., Gambaryan, A., Klimov, A., Castrucci, M. R., Donatelli, I., & Kawaoka, Y. (2000). Early alterations of the receptor-binding properties of H1, H2, and H3 avian influenza virus hemagglutinins after their introduction into mammals. *Journal of Virology*, *74*, 8502–8512. https://doi.org/10.1128/jvi.74.18.8502-8512.2000

Matrosovich, M. N., Gambaryan, A. S., Teneberg, S., Piskarev, V. E., Yamnikova, S. S., Lvov, D. K., Robertson, J. S., & Karlsson, K. A. (1997). Avian influenza a viruses differ from human viruses by recognition of sialyloligosaccharides and gangliosides and by a higher conservation of the HA receptor-binding site. *Virology*, *233*, 224–234. https://doi.org/10.1006/viro.1997.8580

Meijer, A., Bosman, A., van de Kamp, E. E. H. M., Wilbrink, B., Ry, D., van Beest, H. M., & Koopmans, M. (2006). Measurement of antibodies to avian influenza virus a(H7N7) in humans by hemagglutination inhibition test. *Journal of Virological Methods*, *132*(1–2), 113–120. https://doi.org/10.1016/j.jviromet.2005.10.001

Mellis, A. M., Coyle, J., Marshall, K. E., Frutos, A. M., Singleton, J., Drehoff, C., Merced-Morales, A., Pagano, H. P., Alade, R. O., White, E. B., Noble, E. K., Holiday, C., Liu, F., Jefferson, S., Li, Z.-N., Gross, F. L., Olsen, S. J., Dugan, V. G., Reed, C., … Levine, M. Z. (2024). Serologic evidence of recent infection with highly pathogenic avian influenza a(H5) virus among dairy workers - Michigan and Colorado, June-august 2024. *MMWR. Morbidity and Mortality Weekly Report*, *73*, 1004–1009. https://doi.org/10.15585/mmwr.mm7344a3

Millman, A. J., Havers, F., Iuliano, A. D., Davis, C. T., Sar, B., Sovann, L., Chin, S., Corwin, A. L., Vongphrachanh, P., Douangngeun, B., Lindblade, K. A., Chittaganpitch, M., Kaewthong, V., Kile, J. C., Nguyen, H. T., Pham, D. V., Donis, R. O., & Widdowson, M.-A. (2015). Detecting spread of avian influenza a(H7N9) virus beyond China. *Emerging Infectious Diseases*, *21*, 741–749. https://doi.org/10.3201/eid2105.141756

Moreno, A., Bonfante, F., Bortolami, A., Cassaniti, I., Caruana, A., Cottini, V., Cereda, D., Farioli, M., Fusaro, A., Lavazza, A., Lecchini, P., Lelli, D., Maroni Ponti, A., Nassuato, C., Pastori, A., Rovida, F., Ruocco, L., Sordilli, M., Baldanti, F., & Terregino, C. (2023). Asymptomatic infection with clade 2.3.4.4b highly pathogenic avian influenza a(H5N1) in carnivore pets, Italy, April 2023. *Eurosurveillance*, *28*, 2300441. https://doi.org/10.2807/1560-7917.ES.2023.28.35.2300441

Murawski, A., Fabrizio, T., Ossiboff, R., Kackos, C., Jeevan, T., Jones, J. C., Kandeil, A., Walker, D., Turner, J. C. M., Patton, C., Govorkova, E. A., Hauck, H., Mickey, S., Barbeau, B., Bommineni, Y. R., Torchetti, M., Lantz, K., Kercher, L., Allison, A. B., … Webby, R. J. (2024). Highly pathogenic avian influenza a(H5N1) virus in a common bottlenose dolphin (Tursiops truncatus) in Florida. *Communications Biology*, *7*, 476. https://doi.org/10.1038/s42003–024–06173-x

Muthuri, S. G., Myles, P. R., Venkatesan, S., Leonardi-Bee, J., & Nguyen-Van-Tam, J. S. (2013). Impact of neuraminidase inhibitor treatment on outcomes of public health importance during the 2009–2010 influenza a(H1N1) pandemic: A systematic review and meta-analysis in hospitalized patients. *Journal of Infectious Diseases*, *207*, 553–563. https://doi.org/10.1093/infdis/jis726

NIH (National Institutes of Health). (online). High H5N1 influenza levels found in mice given raw milk from infected dairy cows. https://www.nih.gov/news-events/news-releases/high-h5n1-influenza-levels-found-mice-given-raw-milk-infected-dairy-cows

Offeddu, V., Cowling, B. J., & Malik Peiris, J. S. (2016). Interventions in live poultry markets for the control of avian influenza: A systematic review. *One Health*, *2*, 55–64. https://doi.org/10.1016/j.onehlt.2016.03.002

OFFLU. (2024). OFFLU Avian Influenza vaccine Matching (AIM) for poultry vaccines: H5Nx executive summary. https://www.offlu.org/wp-content/uploads/2024/11/OFFLU-Avian-Influenza-Vaccine-Matching-final-clean.pdf

Parry, J. (2005). Use of antiviral drug in poultry is blamed for drug resistant strains of avian flu. *British Medical Journal*, *331*(7507), 10. https://doi.org/10.1136/bmj.331.7507.10

Peacock, T., Moncla, L., Dudas, G., VanInsberghe, D., Sukhova, K., Lloyd-Smith, J. O., Worobey, M., Lowen, A. C., & Nelson, M. I. (2024). The global H5N1 influenza panzootic in mammals. *Nature*, *637*, 304–313. https://doi.org/10.1038/s41586–024–08054-z

Pinto, R. M., Bakshi, S., Lytras, S., Zakaria, M. K., Swingler, S., Worrell, J. C., Herder, V., Hargrave, K. E., Varjak, M., Cameron-Ruiz, N., Collados Rodriguez, M., Varela, M., Wickenhagen, A., Loney, C., Pei, Y., Hughes, J., Valette, E., Turnbull, M. L., Furnon, W., … Palmarini, M. (2023). BTN3A3 evasion promotes the zoonotic potential of influenza a viruses. *Nature*, *619*(7969), 338–347. https://doi.org/10.1038/s41586-023-06261-8

Plaza, P. I., Gamarra-Toledo, V., Rodríguez Euguí, J. R., Rosciano, N., & Lambertucci, S. A. (2024). Pacific and Atlantic sea lion mortality caused by highly pathogenic avian influenza a(H5N1) in South America. *Travel Medicine and Infectious Disease*, *59*, 102712. https://doi.org/10.1016/j.tmaid.2024.102712

Poovorawan, Y., Pyungporn, S., Prachayangprecha, S., & Makkoch, J. (2013). Global alert to avian influenza virus infection: From H5N1 to H7N9. *Pathogens and Global Health*, *107*, 217–223. https://doi.org/10.1179/2047773213Y.0000000103

Puglia, I., Caporale, M., Mangone, I., Petrova, T., Castelli, P., Radomski, N., Ruvo, A. D., Pasquale, A. D., & Lorusso, A. (2024). Analysis of zoonotic mutations of avian influenza viruses. https://zenodo.org/records/14024886

Puryear, W., Sawatzki, K., Hill, N., Foss, A., Stone, J. J., Doughty, L., Walk, D., Gilbert, K., Murray, M., Cox, E., Patel, P., Mertz, Z., Ellis, S., Taylor, J., Fauquier, D., Smith, A., DiGiovanni, R. A., van de Guchte, A., Gonzalez-Reiche, A. S., … Runstadler, J. (2023). Highly pathogenic avian influenza a(H5N1) virus outbreak in new England seals, United States. *Emerging Infectious Diseases*, *29*, 786–791. https://doi.org/10.3201/eid2904.221538

Puzelli, S., Rossini, G., Facchini, M., Vaccari, G., Di Trani, L., Di Martino, A., Gaibani, P., Vocale, C., Cattoli, G., Bennett, M., McCauley, J. W., Rezza, G., Moro, M. L., Rangoni, R., Finarelli, A. C., Landini, M. P., Castrucci, M. R., Donatelli, I., & Influenza Task Force. (2014). Human infection with highly pathogenic a(H7N7) avian influenza virus, Italy, 2013. *Emerging Infectious Diseases*, *20*, 1745–1749. https://doi.org/10.3201/eid2010.140512

Qi, Y., Guo, W., Liu, C., Li, W., Gu, Y., Li, S., & Chen, X. (2021). Seroprevalence of influenza a (H9N2) virus infection among humans in China: A meta-analysis. *Microbial Pathogenesis*, *155*, 104881. https://doi.org/10.1016/j.micpath.2021.104881

Rabalski, L., Milewska, A., Pohlmann, A., Gackowska, K., Lepionka, T., Szczepaniak, K., Swiatalska, A., Sieminska, I., Arent, Z., Beer, M., Koopmans, M., Grzybek, M., & Pyrc, K. (2023). Emergence and potential transmission route of avian influenza a (H5N1) virus in domestic cats in Poland, June 2023. *Eurosurveillance*, *28*, 2300390. https://doi.org/10.2807/1560–7917.ES.2023.28.31.2300390

Racicot, M., Venne, D., Durivage, A., & Vaillancourt, J.-P. (2011). Description of 44 biosecurity errors while entering and exiting poultry barns based on video surveillance in Quebec, Canada. *Preventive Veterinary Medicine*, *100*(3–4), 193–199. https://doi.org/10.1016/j.prevetmed.2011.04.011

Randall, N. J., Blitvich, B. J., & Blanchong, J. A. (2012). Efficacy of wildlife rehabilitation centers in surveillance and monitoring of pathogen activity: A case study with West Nile virus. *Journal of Wildlife Diseases*, *48*, 646–653. https://doi.org/10.7589/0090-3558-48.3.646

Rodriguez, Z., Picasso-Risso, C., O'Connor, A., & Ruegg, P. L. (2024). Hot topic: Epidemiological and clinical aspects of highly pathogenic avian influenza H5N1 in dairy cattle. *JDS Communications*, *5*(Suppl 1), S8–S12. https://doi.org/10.3168/jdsc.2024-0650

Romero Tejeda, A., Aiello, R., Salomoni, A., Berton, V., Vascellari, M., & Cattoli, G. (2015). Susceptibility to and transmission of H5N1 and H7N1 highly pathogenic avian influenza viruses in bank voles (*Myodes glareolus*). *Veterinary Research*, *46*, 51. https://doi.org/10.1186/s13567-015-0184-1

Root, J. J., Bosco-Lauth, A. M., Marlenee, N. L., & Bowen, R. A. (2018). Cottontail rabbits shed clade 2.3.4.4 H5 highly pathogenic avian influenza a viruses. *Archives of Virology*, *163*, 2823–2827. https://doi.org/10.1007/s00705–018–3907–6

Russell, C. J., Hu, M., & Okda, F. A. (2018). Influenza hemagglutinin protein stability, activation, and pandemic risk. *Trends in Microbiology*, *26*, 841–853. https://doi.org/10.1016/j.tim.2018.03.005

Sah, R., Srivastava, S., Kumar, S., Mehta, R., Donovan, S., Sierra-Carrero, L., Luna, C., Woc-Colburn, L., Cardona-Ospina, J. A., & Hinestroza-Jordan, M. (2024). Concerns on H5N1 avian influenza given the outbreak in US dairy cattle. *The Lancet Regional Health Americas*, *35*, 100785. https://doi.org/10.1016/j.lana.2024.100785

Santos, J. D., Sobral, D., Pinheiro, M., Isidro, J., Bogaardt, C., Pinto, M., Eusébio, R., Santos, A., Mamede, R., Horton, D. L., Gomes, J. P., TELEVIR Consortium, & Borges, V. (2024). INSaFLU-TELEVIR: An open web-based bioinformatics suite for viral metagenomic detection and routine genomic surveillance. *Genome Medicine*, *16*, 61. https://doi.org/10.1186/s13073-024-01334-3

Schrauwen, E. J., de Graaf, M., Herfst, S., Rimmelzwaan, G. F., Osterhaus, A. D., & Fouchier, R. A. (2014). Determinants of virulence of influenza a virus. *European Journal of Clinical Microbiology and Infectious Diseases*, *33*, 479–490. https://doi.org/10.1007/s10096-013-1984-8

Shinya, K., Ebina, M., Yamada, S., Ono, M., Kasai, N., & Kawaoka, Y. (2006). Avian flu: Influenza virus receptors in the human airway. *Nature*, *440*(7083), 435–436. https://doi.org/10.1038/440435a

Shittu, I., Silva, D., Oguzie, J. U., Marushchak, L. V., Olinger, G. G., Lednicky, J. A., Trujillo-Vargas, C. M., Schneider, N. E., Hao, H., & Gray, G. C. (2024). A one health investigation into H5N1 avian influenza virus epizootics on two dairy farms. *medRxiv*. https://doi.org/10.1101/2024.07.27.24310982

Shriner, S. A., & Root, J. J. (2020). A review of avian influenza a virus associations in synanthropic birds. *Viruses*, *12*, 1209. https://doi.org/10.3390/v12111209

Shriner, S. A., VanDalen, K. K., Mooers, N. L., Ellis, J. W., Sullivan, H. J., Root, J. J., Pelzel, A. M., & Franklin, A. B. (2012). Low-pathogenic avian influenza viruses in wild house mice. *PLoS One*, *7*, e39206. https://doi.org/10.1371/journal.pone.0039206

Shu, Y., & McCauley, J. (2017). GISAID: Global initiative on sharing all influenza data - from vision to reality. *Eurosurveillance*, *22*, 30494. https://doi.org/10.2807/1560-7917.ES.2017.22.13.30494

Sidney, J., Kim, A. R., de Vries, R. D., Peters, B., Meade, P. S., Krammer, F., Grifoni, A., & Sette, A. (2024). Targets of influenza human T cell response are mostly conserved in H5N1. *bioRxiv*. https://doi.org/10.1101/2024.09.09.612060

Siegers, J. Y., Xie, R., Byrne, A. M. P., Edwards, K. M., Hu, S., Yann, S., Sin, S., Tok, S., Chea, K., Horm, S., Rith, C., Keo, S., Pum, L., Duong, V., Auerswald, H., Phou, Y., Kol, S., Spiegel, A., & Harvey, R. (2024). Emergence of a novel reassortant clade 2.3.2.1c avian influenza a/H5N1 virus associated with human cases in Cambodia. *medRxiv*. https://doi.org/10.1101/2024.11.04.24313747

Sillman, S. J., Drozd, M., Loy, D., & Harris, S. P. (2023). Naturally occurring highly pathogenic avian influenza virus H5N1 clade 2.3.4.4b infection in three domestic cats in North America during 2023. *Journal of Comparative Pathology*, *205*, 17–23. https://doi.org/10.1016/j.jcpa.2023.07.001

Skowronski, D. M., Chambers, C., Gustafson, R., Purych, D. B., Tang, P., Bastien, N., Krajden, M., & Li, Y. (2016). Avian influenza a(H7N9) virus infection in 2 travelers returning from China to Canada, January 2015. *Emerging Infectious Diseases*, *22*, 71–74. https://doi.org/10.3201/eid2201.151330

Songserm, T., Amonsin, A., Jam-on, R., Sae-Heng, N., Pariyothorn, N., Payungporn, S., Theamboonlers, A., Chutinimitkul, S., Thanawongnuwech, R., & Poovorawan, Y. (2006). Fatal avian influenza A H5N1 in a dog. *Emerging Infectious Diseases*, *12*, 1744–1747. https://doi.org/10.3201/eid1211.060542

Sreenivasan, C. C., Thomas, M., Kaushik, R. S., Wang, D., & Li, F. (2019). Influenza a in bovine species: A narrative literature review. *Viruses*, *11*, 561. https://doi.org/10.3390/v11060561

Sun, L., Zhou, P., He, S., Luo, Y., Jia, K., Fu, C., Sun, Y., He, H., Tu, L., Ning, Z., Yuan, Z., Wang, H., Li, S., & Yuan, L. (2015). Sparse serological evidence of H5N1 avian influenza virus infections in domestic cats, northeastern China. *Microbial Pathogenesis*, *82*, 27–30. https://doi.org/10.1016/j.micpath.2015.03.003

Suttie, A., Deng, Y.-M., Greenhill, A. R., Dussart, P., Horwood, P. F., & Karlsson, E. A. (2019). Inventory of molecular markers affecting biological characteristics of avian influenza a viruses. *Virus Genes*, *55*, 739–768. https://doi.org/10.1007/s11262-019-01700-z

Szablewski, C. M., Iwamoto, C., Olsen, S. J., Greene, C. M., Duca, L. M., Davis, C. T., Coggeshall, K. C., Davis, W. W., Emukule, G. O., Gould, P. L., Fry, A. M., Wentworth, D. E., Dugan, V. G., Kile, J. C., & Azziz-Baumgartner, E. (2023). Reported global avian influenza detections among humans and animals during 2013–2022: Comprehensive review and analysis of available surveillance data. *JMIR Public Health and Surveillance*, *9*, e46383. https://doi.org/10.2196/46383

Tammiranta, N., Isomursu, M., Fusaro, A., Nylund, M., Nokireki, T., Giussani, E., Zecchin, B., Terregino, C., & Gadd, T. (2023). Highly pathogenic avian influenza a (H5N1) virus infections in wild carnivores connected to mass mortalities of pheasants in Finland. *Infection, Genetics and Evolution*, *111*, 105423. https://doi.org/10.1016/j.meegid.2023.105423

te Beest, D. E., van Boven, M., Bos, M. E. H., Stegeman, A., & Koopmans, M. P. G. (2010). Effectiveness of personal protective equipment and oseltamivir prophylaxis during avian influenza a (H7N7) epidemic, The Netherlands, 2003. *Emerging Infectious Diseases*, *16*, 1562–1568. https://doi.org/10.3201/eid1610.091412

Tesini, B. L., Kanagaiah, P., Wang, J., Hahn, M., Halliley, J. L., Chaves, F. A., Nguyen, P. Q. T., Nogales, A., DeDiego, M. L., Anderson, C. S., Ellebedy, A. H., Strohmeier, S., Krammer, F., Yang, H., Bandyopadhyay, S., Ahmed, R., Treanor, J. J., Martinez-Sobrido, L., Golding, H., … Sangster, M. Y. (2019). Broad hemagglutinin-specific memory B cell expansion by seasonal influenza virus infection reflects early-life imprinting and adaptation to the infecting virus. *Journal of Virology*, *93*, e00169–19. https://doi.org/10.1128/JVI.00169-19

Tharakaraman, K., Raman, R., Viswanathan, K., Stebbins, N. W., Jayaraman, A., Krishnan, A., Sasisekharan, V., & Sasisekharan, R. (2013). Structural determinants for naturally evolving H5N1 hemagglutinin to switch its receptor specificity. *Cell*, *153*, 1475–1485. https://doi.org/10.1016/j.cell.2013.05.035

Tisza, M. J., Hanson, B. M., Clark, J. R., Wang, L., Payne, K., Ross, M. C., Mena, K. D., Gitter, A., Javornik Cregeen, S. J., Cormier, J., Avadhanula, V., Terwilliger, A., Balliew, J., Wu, F., Rios, J., Deegan, J., Piedra, P. A., Petrosino, J. F., Boerwinkle, E., & Maresso, A. W. (2024). Sequencing-based detection of avian influenza a(H5N1) virus in wastewater in ten cities. *New England Journal of Medicine*, *391*, 1157–1159. https://doi.org/10.1056/NEJMc2405937

Tiwari, A., Meriläinen, P., Lindh, E., Kitajima, M., Österlund, P., Ikonen, N., Savolainen-Kopra, C., & Pitkänen, T. (2024). Avian influenza outbreaks: Human infection risks for beach users - one health concern and environmental surveillance implications. *Science of the Total Environment*, *943*, 173692. https://doi.org/10.1016/j.scitotenv.2024.173692

Uiprasertkul, M., Puthavathana, P., Sangsiriwut, K., Pooruk, P., Srisook, K., Peiris, M., Nicholls, J. M., Chokephaibulkit, K., Vanprapar, N., & Auewarakul, P. (2005). Influenza a H5N1 replication sites in humans. *Emerging Infectious Diseases*, *11*, 1036–1041. https://doi.org/10.3201/eid1107.041313

Ulloa, M., Fernández, A., Ariyama, N., Colom-Rivero, A., Rivera, C., Nuñez, P., Sanhueza, P., Johow, M., Araya, H., Torres, J. C., Gomez, P., Muñoz, G., Agüero, B., Alegría, R., Medina, R., Neira, V., & Sierra, E. (2023). Mass mortality event in south American sea lions (*Otaria flavescens*) correlated to highly pathogenic avian influenza (HPAI) H5N1 outbreak in Chile. *Veterinary Quarterly*, *43*, 1–10. https://doi.org/10.1080/01652176.2023.2265173

USDA (United States Department of Agriculture). (2024a). Center for Veterinary Biologics Notice No. 24-13. https://www.aphis.usda.gov/sites/default/files/notice24-13.pdf

USDA (United States Department of Agriculture). (2024b). Highly pathogenic avian influenza (H5N1) – Michigan dairy herd and poultry flock summary. Epidemiological investigations of HPAI H5N1 genotype B3.13 in Michigan dairy herds and poultry flocks. https://www.aphis.usda.gov/sites/default/files/hpai-h5n1-dairy-cattle-mi-epi-invest.pdf

USDA (United States Department of Agriculture). (2024c). Highly pathogenic avian influenza H5N1 genotype B3.13 in dairy cattle: National Epidemiologic Brief. U.S. Department of Agriculture. https://www.aphis.usda.gov/sites/default/files/hpai-dairy-national-epi-brief.pdf

USDA (United States Department of Agriculture). (online-a). Detections of highly pathogenic avian influenza (HPAI) in livestock. https://www.aphis.usda.gov/livestock-poultry-disease/avian/avian-influenza/hpai-livestock

USDA (United States Department of Agriculture). (online-b). Detections of highly pathogenic avian influenza in mammals. https://www.aphis.usda.gov/livestock-poultry-disease/avian/avian-influenza/hpai-detections/mammals

USDA (United States Department of Agriculture). (online-c). USDA Animal and Plant Health Inspection Service shares update on H5N1 detection in Oregon swine, bovine vaccine candidate progression. https://www.aphis.usda.gov/news/agency-announcements/usda-animal-plant-health-inspection-service-shares-update-h5n1-detection

Van Kerkhove, M. D., Mumford, E., Mounts, A. W., Bresee, J., Ly, S., Bridges, C. B., & Otte, J. (2011). Highly pathogenic avian influenza (H5N1): Pathways of exposure at the animal-human interface, a systematic review. *PLoS One*, *6*, e14582. https://doi.org/10.1371/journal.pone.0014582

Velkers, F. C., Blokhuis, S. J., Veldhuis Kroeze, E. J. B., & Burt, S. A. (2017). The role of rodents in avian influenza outbreaks in poultry farms: A review. *Veterinary Quarterly*, *37*, 182–194. https://doi.org/10.1080/01652176.2017.1325537

Wang, J., Wu, M., Hong, W., Fan, X., Chen, R., Zheng, Z., Zeng, Y., Huang, R., Zhang, Y., Lam, T. T., Smith, D. K., Zhu, H., & Guan, Y. (2016). Infectivity and transmissibility of avian H9N2 influenza viruses in pigs. *Journal of Virology*, *90*, 3506–3514. https://doi.org/10.1128/JVI.02605-15

Wang, W., Chen, X., Wang, Y., Lai, S., Yang, J., Cowling, B. J., Horby, P. W., Uyeki, T. M., & Yu, H. (2022). Serological evidence of human infection with avian influenza a(H7N9) virus: A systematic review and meta-analysis. *Journal of Infectious Diseases*, *226*, 70–82. https://doi.org/10.1093/infdis/jiaa679

Wang, X., Wu, P., Pei, Y., Tsang, T. K., Gu, D., Wang, W., Zhang, J., Horby, P. W., Uyeki, T. M., Cowling, B. J., & Yu, H. (2019). Assessment of human-to-human transmissibility of avian influenza a(H7N9) virus across 5 waves by analyzing clusters of case patients in mainland China, 2013–2017. *Clinical Infectious Diseases*, *68*, 623–631. https://doi.org/10.1093/cid/ciy541

Wang, Y., Yang, C., Liu, Y., Zhang, J., Qu, W., Liang, J., Tu, C., Mai, Q., Mai, K., Feng, P., Huang, W., Lin, Z., Hon, C., Yang, Z., & Pan, W. (2024). Seroprevalence of avian influenza a(H5N6) virus infection, Guangdong Province, China, 2022. *Emerging Infectious Diseases*, *30*, 826–828. https://doi.org/10.3201/eid3004.231226

Webby, R. J., & Uyeki, T. M. (2024). An update on highly pathogenic avian influenza a(H5N1) virus, clade 2.3.4.4b. *Journal of Infectious Diseases*, *230*, 533–542. https://doi.org/10.1093/infdis/jiae379

WHO (World Health Organization). (2018). WHO information for the molecular detection of influenza viruses. https://cdn.who.int/media/docs/default-source/influenza/global-influenza-surveillance-and-response-system/related-documents/protocols_influenza_virus_detection_nov_2018.pdf?sfvrsn=60fae07e_10

WHO (World Health Organization). (2020). Tool for Influenza Pandemic Risk Assessment (TIPRA). 2nd edition. Version 2. Release 31. https://cdn.who.int/media/docs/default-source/influenza/tipra_guidance_version-2_20211008.pdf?sfvrsn=88132b6c_6&download=true

WHO (World Health Organization). (2024). Guidance on selection and use of one health operational tools. https://iris.who.int/bitstream/handle/10665/376977/9789290211426-eng.pdf?sequence=1

WHO (World Health Organization). (online). WHO H5 Reference Labs. https://www.who.int/initiatives/global-influenza-surveillance-and-response-system/h5-reference-laboratories

WOAH (World Organisation for Animal Health). (2024). Biosecurity procedures in poultry production. https://www.woah.org/en/what-we-do/standards/codes-and-manuals/terrestrial-code-online-access/?id=169&L=1&htmfile=chapitre_biosecu_poul_production.htm

Wolfe, N. D., Dunavan, C. P., & Diamond, J. (2007). Origins of major human infectious diseases. *Nature*, *447*(7142), 279–283. https://doi.org/10.1038/nature05775

Yamaji, R., Zhang, W., Kamata, A., Adlhoch, C., Swayne, D. E., Pereyaslov, D., Wang, D., Neumann, G., Pavade, G., Barr, I. G., Peiris, M., Webby, R. J., Fouchier, R. A. M., Von Dobschütz, S., Fabrizio, T., Shu, Y., & Samaan, M. (2024). Pandemic risk characterisation of zoonotic influenza a viruses using the tool for influenza pandemic risk assessment (TIPRA). *Lancet Microbe*, 100973. https://doi.org/10.1016/j.lanmic.2024.100973

Yang, W., Schountz, T., & Ma, W. (2021). Bat influenza viruses: Current status and perspective. *Viruses*, *13*, 547. https://doi.org/10.3390/v13040547

Yong-Feng, Z., Fei-Fei, D., Jia-Yu, Y., Feng-Xia, Z., Chang-qing, J., Jian-Li, W., Shou-Yu, G., Kai, C., Chuan-Yi, L., Xue-hua, W., Jiang, S.-J., & Zhi-Jing, X. (2017). Intraspecies and interspecies transmission of mink H9N2 influenza virus. *Scientific Reports*, *7*, 7429. https://doi.org/10.1038/s41598-017-07879-1

Yuk, S.-S., Lee, D.-H., Park, J.-K., Tseren-Ochir, E.-O., Kwon, J.-H., Noh, J.-Y., & Song, C.-S. (2017). Experimental infection of dogs with highly pathogenic avian influenza virus (H5N8). *Journal of Veterinary Science*, *18*(Suppl 1), 381–384. https://doi.org/10.4142/jvs.2017.18.S1.381

Zhang, X., Xu, G., Wang, C., Jiang, M., Gao, W., Wang, M., Sun, H., Sun, Y., Chang, K.-C., Liu, J., & Pu, J. (2017). Enhanced pathogenicity and neurotropism of mouse-adapted H10N7 influenza virus are mediated by novel PB2 and NA mutations. *Journal of General Virology*, *98*, 1185–1195. https://doi.org/10.1099/jgv.0.000770

Zhao, J., Wan, W., Yu, K., Lemey, P., Pettersson, J. H.-O., Bi, Y., Lu, M., Li, X., Chen, Z., Zheng, M., Yan, G., Dai, J., Li, Y., Haerheng, A., He, N., Tu, C., Suchard, M. A., Holmes, E. C., He, W.-T., & Su, S. (2024). Farmed fur animals harbour viruses with zoonotic spillover potential. *Nature*, *634*(8032), 228–233. https://doi.org/10.1038/s41586–024–07901–3

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ANNEX A

Protocol for the assessment of risk posed by AI viruses and measures for preparedness, prevention and control

Background and ToRs as provided by the requestor

See Section 1.1.

Problem formulation

Here, a summary of the initial considerations is described. With this mandate, the EC requests that ECDC and EFSA provide scientific advice and guidance tools to enhance preparedness, prevention and control measures to better manage the risks pertaining to animal and public health due to the current and potentially evolving avian influenza (AI) situation.

1. For the assessment, the following problems have been addressed:

    a. Quantification of the risk of mutation: assessment of the zoonotic risk posed by different AI subtypes/clades/genotypes in humans by assessing key mutations that may enhance adaptation to mammals, particularly humans.
    b. Assessment of frequency and types of mutations in different subtypes considering connected epidemiological data (animal species, geography, conditions, etc.).
    c. The above (a, b) will be based on an analysis of the zoonotic mutations in the published sequences of all distinct AI virus (AIV) subtypes.
    d. Identification of the factors that influence the mutation frequency of AIVs in birds and mammals towards zoonotic virus.
    e. Analysis of human case epidemiological data and review of human seroprevalence studies.
    f. **Assessment of surveillance tools with a view of addressing zoonotic risks:** The focus should be on the surveillance to detect zoonotic mutation in birds and mammals, and which species most at risk for spillover should be targeted. This should focus on reliable and feasible schemes for Member States (MSs).
    g. A global perspective should be taken, not only for the EU.
    h. Human surveillance is included.

2. To provide a comprehensive set of prevention, protection and preparedness measures:

    a. Expand on specific points addressed in the report on task 1 of the mandate.
    b. Biosecurity at the farm: tailor recommendations to the farm environment.
    c. Focus on occupationally exposed people.
    d. Targets for the levels of communication would include: the general public; occupationally exposed; backyard poultry farming.

Clarification of the scope of the request: framework, population and geographical area of concern, definitions

- Scope: to provide a framework for assessment of the risk of AIV, focusing on the risk of mutation towards zoonotic strains and complementing with a descriptive analysis of human epidemiological data and human seroprevalence studies and to provide a set of measures for prevention and control.
- Geographical area: EU and worldwide.
- Population: poultry and domestic birds, wild birds, domestic and wild mammals and humans.

Assessing and synthesising evidence (including uncertainty analysis)

- Details of the methodology used for the analysis of the data retrieved by the literature review, as well as data from other sources will be provided in the methodology section of the opinion.
- All sources of uncertainty identified during the assessment are recorded, and their impact on the scientific assessment has been assessed collectively.

ANNEX B

Tables of eligible seroprevalence studies for H5, H7 and H9 avian influenza subtypes (published 2020–2024)

TABLE B.1 Summary of systematic literature review/meta-analysis and additional eligible primary studies with results on seroprevalence of avian influenza H5 published between 2020 and 2024.

Author	Country	Publication year	Study design	Study period	Study population	Demographic data	Method/cut-off titre	Number of tested	Number of seropositive	Seroprevalence %	95% confidence interval	Clinical presentation
Chen et al. (2020)	Multiple countries with H5N1 outbreaks	2020	Systematic review and meta-analysis	Serosurveys published from 1997 to 2020	Mostly exposed individuals	Varying between study	HI and MN. Studies were divided into three groups depending on the cut-off titre: (1) WHO recommended: ≥ 1:80 with a positive result using a second confirmatory assay. (2) WHO modified: titre ≥ 1:80 with a positive result using a second confirmatory assay (i.e. HAI antibody titre ≥ 1:40, ELISA or western blot assay). (3) Criteria used to define seropositive results other than the WHO or modified WHO definitions			(1) Poultry worker: 0.2% Poultry cullers: 0.6% Persons with both poultry and human exposure: 1.8% Close contacts: 0% General population: 0% (2) 0% (3) 0.2% Poultry workers: 0.5% Poultry cullers: 0.4% Persons with exposure to both poultry and human cases: 0.8%	Not given	Both asymptomatic and symptomatic cases are included
Wang et al. (2024)	China	2024	Cross-sectional study	January–March 2022	General population	Median age 41 42.2% male	HI and MN assay. For HI: ≥ 1:10 was seropositive. For MN: ≥ 1:20 was considered seropositive	6363	1	0.02%	Not given	The study included individuals regardless of respiratory symptoms
Chaudhry et al. (2020)	Pakistan	2020	Cross-sectional study	December 2015 to March 2016	Butchers working in live poultry retail stalls	Butchers: Median age was 28 and all were male	HI and MN. ≥ 1:40 for HI and ≥ 1:20 for MN were considered seropositive	161	0	Butchers: 0%		Asymptomatic
	Pakistan	2020	Cross-sectional study	December 2015 to March 2016	A control group from the general population	General population: Median age was 27 and 33% were male	HI and MN. ≥ 1:40 for HI and ≥ 1:20 for MN were considered seropositive	100	0	General population: 0%		Asymptomatic

TABLE B.2 Summary of systematic literature review/meta-analysis and additional eligible primary studies with results on seroprevalence of avian influenza H7 published between 2020 and 2024.

Author	Country	Year	Study design	Study period	Study population	Demographic data	Method/cut-off titre	Number of tested	Number of seropositive	Seroprevalence % (number of tested)	95% confidence interval	Clinical presentation
Wang et al. (2022)	Multiple countries (31 studies)	2022	Systematic review and meta-analysis	2013–2020	Exposed populations and the general population	–	WHO recommended (HAI titre ≥ 160 tested or an HAI titre of 20–80 with a positive result using a second confirmatory assay or western blot assay), modified WHO-recommended and non-standardised definitions	Table 1, individual studies	Table 1, individual studies	Poultry workers, the prevalence of H7N9-specific antibodies was 0.1%, 0.4% and 0.5%, the seroprevalence for close contacts (0.2%), general population 0%	1.7%–8.3%, 0%–0.9%, (0% [95% CI, 0%–0.1%]), respectively	Among 19 studies that assessed participant's respiratory symptoms, the seroprevalence of asymptomatic A(H7N9) virus infections was higher for close contacts (0.2% [95% CI, 0%–0.9%]) and lower for poultry workers (0% [95% CI, 0%–0.1%])
Chaudhry et al. (2020)	Pakistan	2020	Cross-sectional	December 2015 to March 2016	Butchers in live poultry retail stalls	100% males. Age from 15 years old	HI and MN. Titre thresholds: ≥ 1:40 for HI and ≥ 1:20 for MN	161	0	0%	Not given	Asymptomatic
	Pakistan	2020	Cross-sectional	December 2015 to March 2016	Controls: General population has no exposure to poultry or livestock during their daily routine	100% males. Age from 15 years old	HI and MN. Titre thresholds: ≥ 1:40 for HI and ≥ 1:20 for MN	100	0	0%	Not given	Asymptomatic
Horm et al. (2016)	Cambodia	2016	Cohort	January–November 2013	Exposed workers	Adults	HI, MN. HI titre of ≥ 1:80 and a MN titre of ≥ 1:40	111	0	0%	(CI 0.0–3.3)	No poultry workers reported any symptoms in relation to acute avian influenza infection

TABLE B.3 Summary of systematic literature review/meta-analysis and additional eligible primary studies with results on seroprevalence for avian influenza H9 published between 2020 and 2024.

Author	Country	Publication year	Study design	Study period	Study population	Demographic data	Method/cut-off titre	Number of tested	Number of seropositive	Seroprevalence %	95% confidence interval	Clinical presentation
Qi et al. (2021)	China	2021	Systematic review and meta-analysis	1997–2020	Both occupationally exposed and unexposed individuals were included	6029 males, 7920 females, 45,641 unknown. No information about age	HI test was most often used but MN, HI/MN and HI/NT were also used. The seropositive threshold was not mentioned	59,590	3316	The overall seroprevalence was 5.56% Poultry market workers: 4.74%. The combined seroprevalence for all occupationally exposed populations, 3.18%, was higher than the seroprevalence in the general population, 1.38%	Overall: 3.88–7.28	Both asymptomatic and symptomatic cases are included
Chaudhry et al. (2020)	Pakistan	2020	Cross-sectional study	December 2015 to March 2016	Butchers working in live poultry retail stalls	Butchers: Median age was 28 and all were male	HI and MN. ≥ 1:40 for HI and ≥ 1:20 for MN were considered seropositive	161	25	15.6%	95% CI%: 12.1–20.0)	Asymptomatic
	Pakistan	2020	Cross-sectional study	December 2015 to March 2016	A control group from the general population	General population: Median age was 27 and 33% were male	HI and MN. ≥ 1:40 for HI and ≥ 1:20 for MN were considered seropositive	100	6	6%	2.23%–12.60%	Asymptomatic

ANNEX C

Activities and occupations according to the level of exposure

TABLE C.1 List of activities and occupations according to the level of exposure (updated from the 'Investigation protocol for human exposures and cases of avian influenza in the EU/EEA').

Exposure level	Activities and occupations	Type of exposure	Location of exposure	Indoor/outdoor space	Duration of exposure	Viral load
High-level exposure	Occupations or activities that involve direct/indirect contact with an infected animal or its secretions (e.g. inhalation of infected droplets or dust) or close contact allowing for inhalation or ocular exposure to virus droplets without the correct use of appropriate preventive measures or with breach of PPE, e.g. veterinarians/workers with close occupational exposure to animals, individuals involved in cleaning affected premises after culling operations, individuals cleaning areas contaminated with animal faeces or secretions during clean-up and disposal activities, individuals working in backyard holding areas accessible to wild animals, volunteers or other staff handling wild animals or their carcasses, or laboratory workers handling positive AIV specimens without adequate protection. Activities with direct exposure to infected animals, such as slaughtering, butchering and handling infected animals or animal waste without the use of appropriate protective measures (e.g. PPE) or with breach of PPE. The risk of close contact from inhalation of dust/droplets from contaminated clothes/equipment should also be considered. Close contacts (e.g. household members, caretakers, seasonal workers or farm workers who are sharing living areas in agricultural premises, health-care workers caring for a patient with laboratory-confirmed AIV infection without the use of appropriate measures, including PPE or with breach of PPE) or other contacts having encounters with human cases for > 15 min and < 1 m away. Workers may come into contact with raw milk, e.g. at farms or in the dairy industry or in transport/collection/processing of raw milk if there is an A(H5) outbreak in the dairy farm.	Direct/indirect/ocular/inhalation/ingestion	Areas and settings with confirmed AIV outbreaks (e.g. poultry and fur farms)	Indoor spaces with poor ventilation	Prolonged exposure (> 15 min)	High (based on the density of the infected animal)
Moderate-level exposure	Individuals present in infected environments but not directly in contact with sick animals or their secretions, faeces or other body parts (e.g. observers), as well as workers in the occupational groups that include those at high risk of exposure (e.g. farm workers, veterinarians) who do not directly handle infected animals, but who are in their vicinity/of contaminated environments, often without use of appropriate measures. Co-workers of a human AIV infection case share the same workspace, particularly if enclosed, but not having such prolonged close contact as with high-level exposure.	Direct/indirect/ocular/ingestion	Areas with known presence of AIV	Indoor spaces with good ventilation Outdoor contained spaces	Intermediate duration of exposure	Intermediate (based on the density of infected animals)

TABLE C.1 (Continued)

Exposure level	Activities and occupations	Type of exposure	Location of exposure	Indoor/outdoor space	Duration of exposure	Viral load
Low-level exposure	Incidental encounters with sick animals/carcasses or workers in animal-related industries who are not directly in contact with the animal/environment. Exposed individuals should be protected by appropriate measures and correctly using appropriate PPE (e.g. health-care workers), as well as residents in areas with known presence of AIV without direct or indirect contact with sick or dead animals. Incidental encounters with a human case without direct or indirect contact with the human case. For potential contacts during flights, the exposure level should be assessed on a case-by-case basis.	Indirect/ocular/ingestion	Areas with potential, unconfirmed AIV exposure	Outdoor spaces with AIV infections in the area	Brief exposure	Low or unknown

ANNEX D

Supplementary graphs

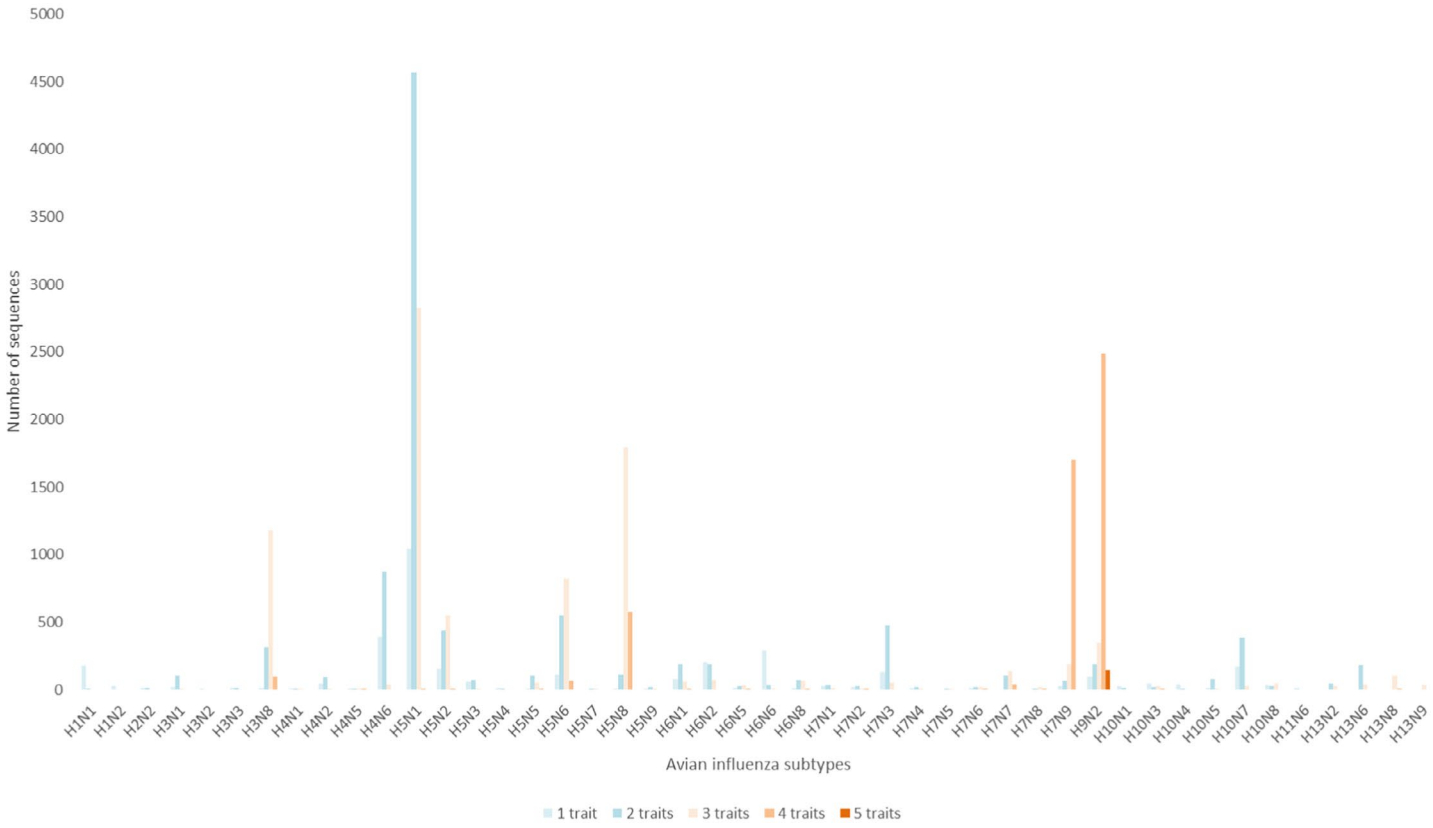

FIGURE D.1 Number of sequences for each influenza subtype by number of traits (1–5) identified in each sequence.

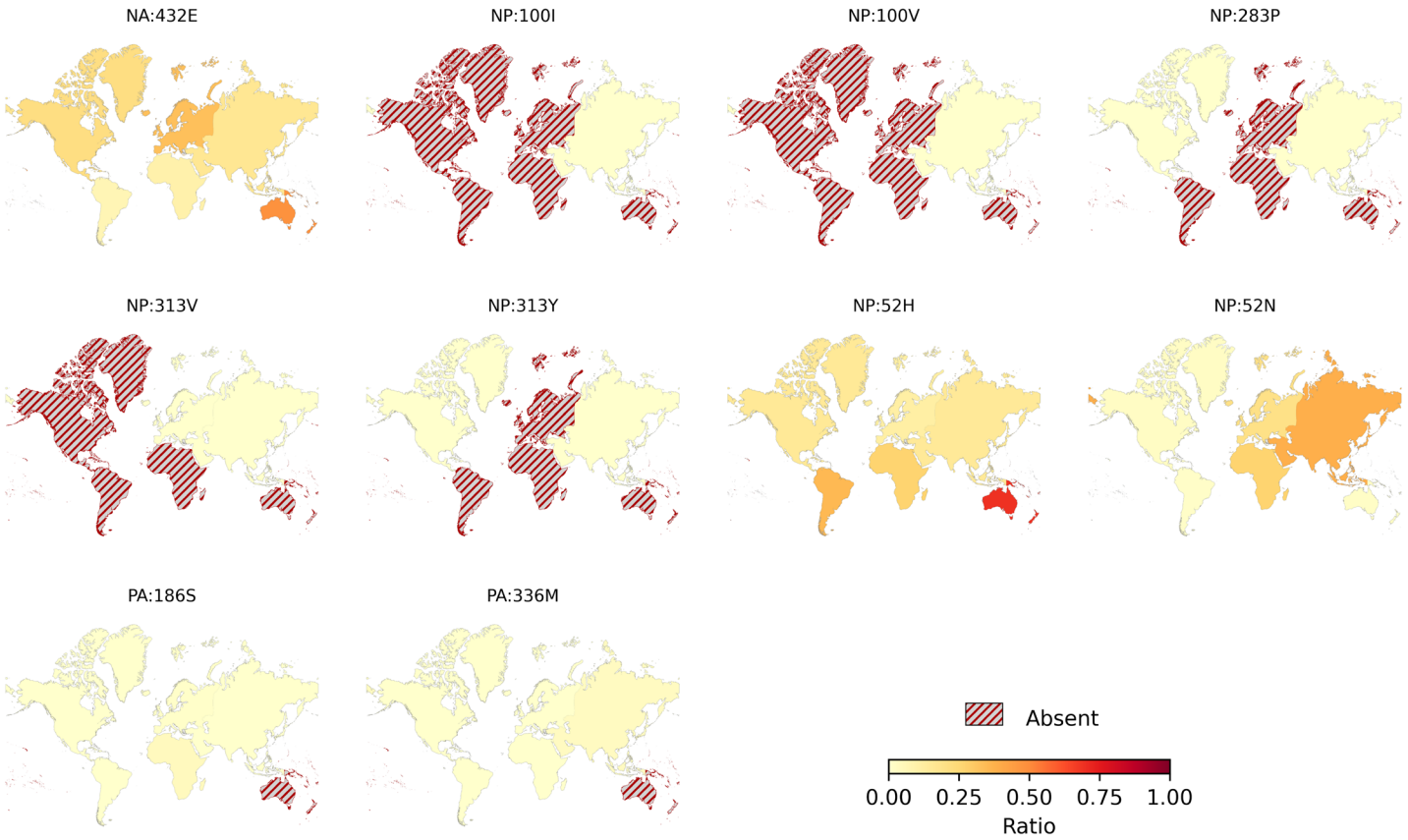

FIGURE D.2 Frequency of shortlisted mutations across continents.

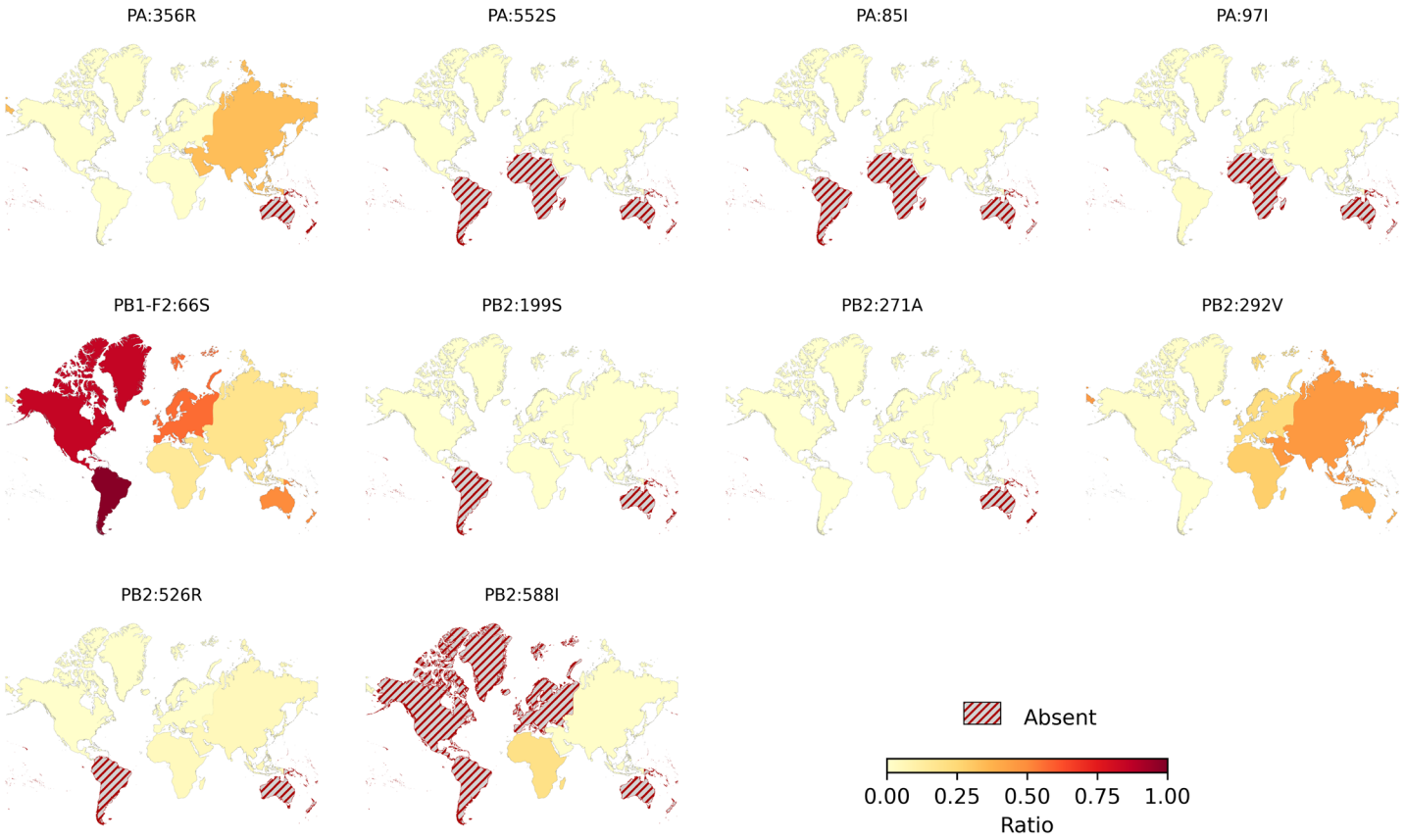

FIGURE D.3 Frequency of shortlisted mutations across continents.

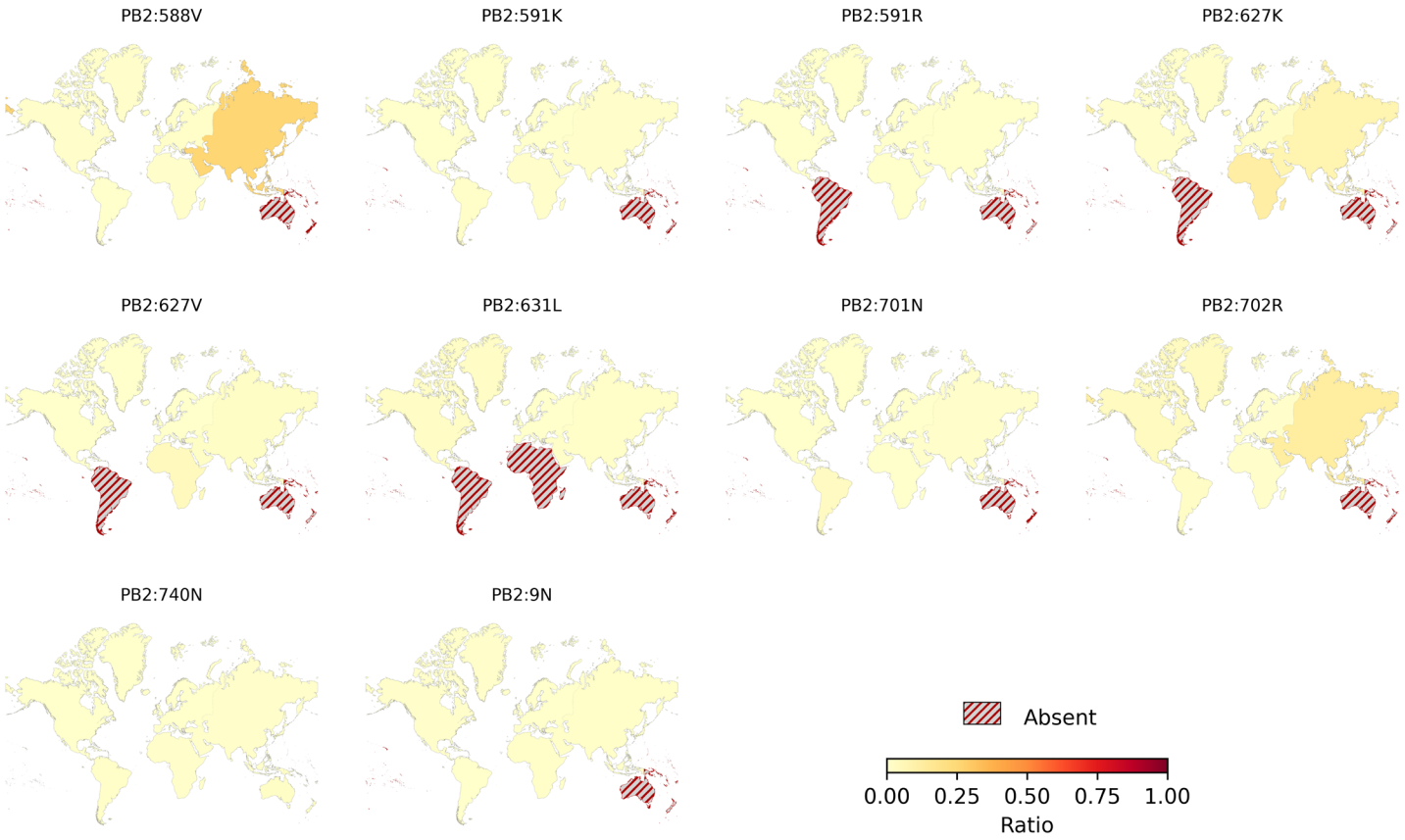

FIGURE D.4 Frequency of shortlisted mutations across continents.

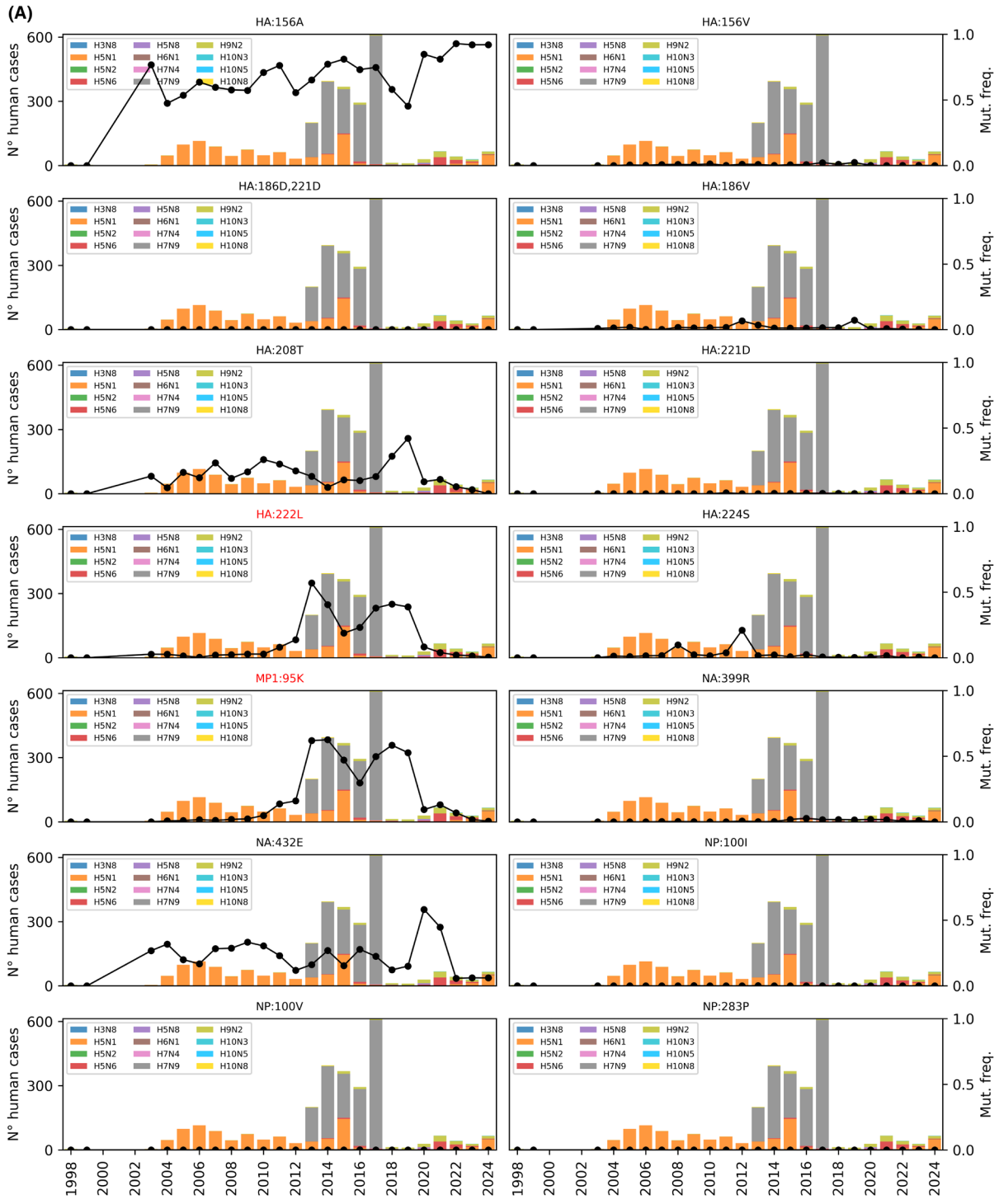

FIGURE D.5 Timeline of the frequency of the 40 mutations (subset 1) in all the analysed viruses plotted over the number of human cases. Each plot represents the timeline (year) of the relative frequency (black line and right *y*-axis) of each of the shortlisted mutations (indicated above each graph). The bars represent the number of AIV human cases reported each year, coloured according to the subtype (left *y*-axis).

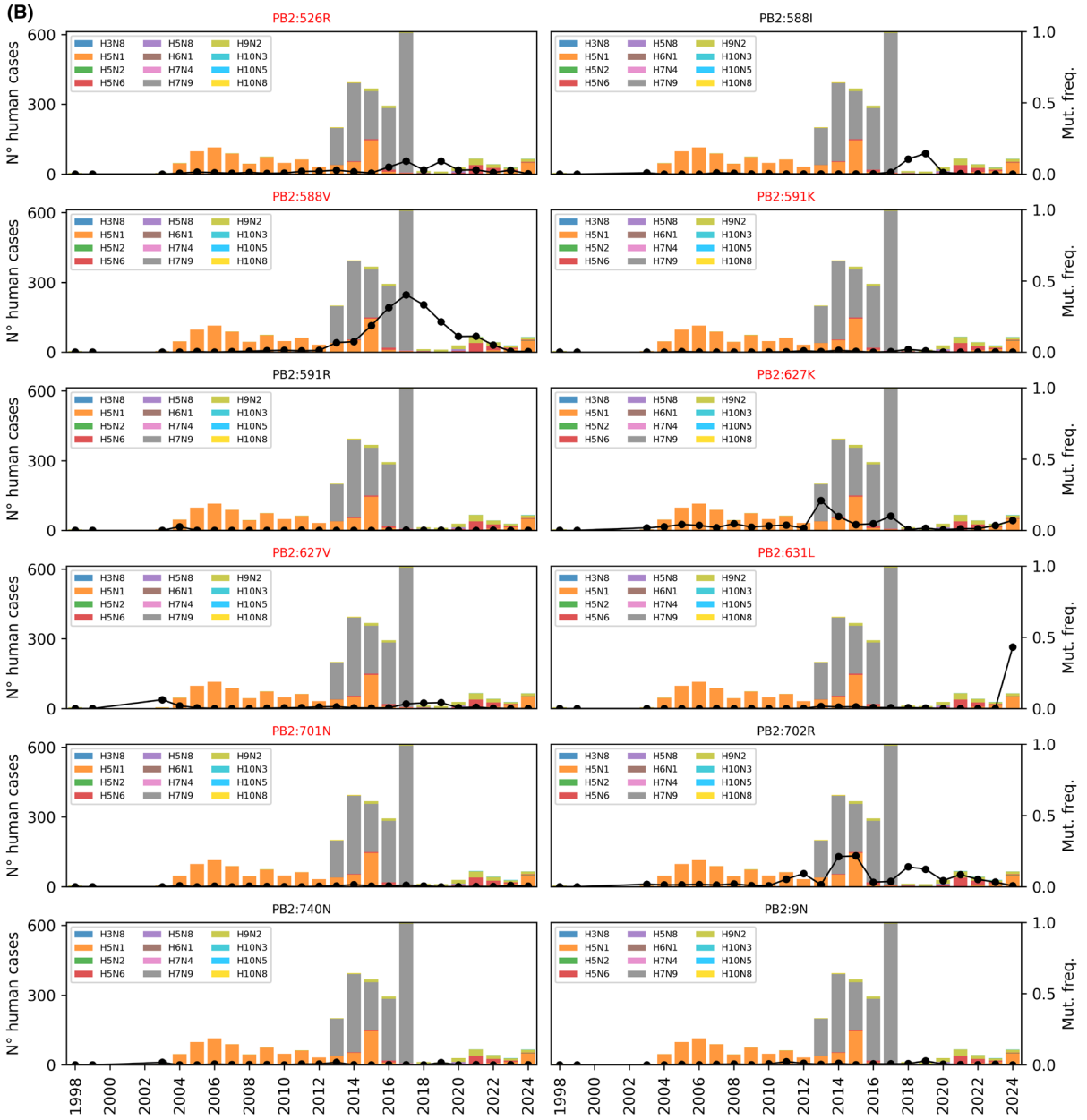

FIGURE D.5 (Continued)