OFFLU Avian Influenza Vaccine Matching (AIM) for poultry vaccines: H5Nx executive summary (October 2024)

Disclaimer: This technical report uses published nomenclature which hasn't recently been updated (widely used by many countries at present (i.e. "GISAID clade")[1] but not recently updated) AND unpublished nomenclature proposed by the WHO/FAO/WOAH H5 Nomenclature Working Group[2] (i.e. provisional Nextclade/LABEL clade). Throughout the report, the use of the different nomenclatures is discriminated by footnotes.

This report provides the point of view of independent OFFLU experts and does not necessarily reflect the position of the parent organisations FAO and WOAH.

Summary

Effectiveness of poultry vaccination against high pathogenicity avian influenza (HPAI) depends on the availability and proper use of suitable vaccines according to age and host species. Antigenic variation in emerging field strains may render existing vaccines less effective. This is particularly true for vaccines that rely primarily on the antibody-based (humoral) immune response (e.g, conventional inactivated vaccines, subunit vaccines). The OFFLU-AIM project's goal is to provide information on the diversity of viral surface proteins (antigenic diversity) in circulating HPAI viruses that could impact the effectiveness of existing vaccines. This project focuses on the goose/Guangdong/1/96-lineage A(H5Nx) viruses (Gd/Gd) which have diversified genetically since their emergence. It is important to highlight that antigenic and genetic evolution are not always concordant (Kok *et al.,* 2024), thus, the information in this report is derived from multiple sources including antigenic testing conducted at FAO and WOAH reference laboratories. Continuous monitoring and full characterisation of viruses, including antibody monitoring, especially in countries using vaccination, are crucial.

The HPAI Gd/Gd clade 2.3.4.4b A(H5N1) viruses are a significant threat to global poultry production. These viruses circulate widely in diverse wild bird species across Eurasia, parts of Africa, and the Americas, with frequent spillovers into poultry and subsequent spread is facilitated by poor biosecurity and poultry movement, with severe impacts to poultry production. Outbreaks in vaccinated poultry have been reported within the past year in France and continuous characterisation of circulating viruses is recommended.

- Clade 2.3.4.4b viruses have replaced other clades in sub-Saharan Africa, with recent viruses indicating some antigenic diversity. Enhanced diagnostic evaluation, and timely release of global data are required to understand vaccine requirements across Africa.
- Clade 2.3.4.4b viruses continue to cause outbreaks in poultry in Europe and circulate in wild birds. Viruses isolated from wild birds have been antigenically stable. Preventative vaccination has been used in ducks in France over winter 2023/2024; however, outbreaks in vaccinated poultry have been reported.

- Outbreaks of avian influenza continue in commercial poultry and backyard flocks in the USA and Canada, predominantly caused by the H5N1 subtype. Limited reports on the antigenic characteristics of recent viruses are not suggestive of antigenic change. In Central and South America, clade 2.3.4.4b viruses were first detected in late 2022, have spread widely, and have disproportionately impacted the backyard poultry sector.
- Clade 2.3.4.4b viruses are widespread in Asia, with multiple incursions into poultry and, in some countries, co-circulation with other-clade viruses. Evidence of antigenic heterogeneity has been observed, highlighting the importance of viral and antigenic monitoring, for the early detection of divergent evolution.
- Clade 2.3.2.1a viruses continue to be detected in South Asia. Recent viruses are genetically and antigenically diverse, suggesting the need for updated vaccine antigens. Conventional inactivated vaccines derived from clade 2.3.4.4 viruses are unlikely to protect against clade 2.3.2.1a viruses, warranting the consideration of multivalent vaccines and heterologous prime-boosting approaches where these clades co-circulate in domestic birds.

There is a close correlation between antibody titre, susceptibility to infection, and disease outcome. When vaccines are used, post-vaccinal antibody responses should be monitored at the farm level to monitor vaccine uptake and mounting of an immune response. If using conventional inactivated vaccines, flocks should be monitored regularly throughout the commercial production cycle. Booster doses should be administered to reduce the likelihood that antibody titres fall below levels expected to prevent disease. Antibody titres are less reliable indicators of protection when using vectored vaccines alone. It is important that viruses detected in vaccinated flocks are characterised and assessed for the presence of mutations that might lead to antigenic changes.

Nothing in this report should be construed as an endorsement of a specific product, and the information provided is general in nature. Additional support on relevant vaccines can be obtained from FAO, WOAH and OFFLU.

Findings

Clade 2.3.4.4b

Antigenic assessments using reference chicken antisera from international partners have shown limited variation in the antigenic characteristics of these 2023 viruses although there was some variation by subtype. This has been noted in viruses from poultry in countries where circulation is presumed enzootic. Tested viruses from wild birds are less antigenically diverse than viruses from poultry. Continuous circulation in wild birds and recurrent spillovers into poultry could further complicate the antigenic profiles of viruses found in some countries. The limited availability of strains from regions where these viruses continue to circulate in poultry has constrained the comprehensiveness of these impartial assessments.

Vaccine efficacy testing has been conducted in several European countries as reported in OFFLU AIM pilot report in a range of poultry hosts. Briefly, coordinated trials assessed different vaccine platforms (inactivated, vectored, recombinant protein, and mRNA vaccines), three domestic host species (chicken, turkey, and duck) and using different vaccination regimens against challenge with contemporary clade 2.3.4.4b viruses. The trials measured clinical outcomes, virus shedding, immune responses and transmission. Overall vaccine selection and vaccination regimens used may need modulating by species to ensure a protective response, for example, data generated from trials on chickens should not be interpreted to infer efficacy of vaccines in other species. (EFSA AHAW Panel, 2023). For more information refer to the EFSA publication (EFSA AHAW Panel, 2023) that summarises the trial data which is not yet available in peer-reviewed literature but via some online reports (Germeraad *et al.,* 2023; Grasland e*t al.,* 2023).

In other vaccine challenge studies carried out in Egypt, local clade 2.3.4.4b H5N1 and H5N8 viruses collected in 2022 and 2019 were used, respectively, as challenge viruses (Hamdy *et al.,* 2024). Three different inactivated vaccines were tested in commercial broiler chickens: an H5N1 clade 2.3.2; an H5N1 clade 2.3.4 and an H5N8 clade 2.3.4.4b. Only the clade 2.3.4.4b vaccine provided clinical protection against experimental challenge with the clade 2.3.4.4b H5N1 2022 virus 21 days post-single vaccination. No information was available on viral shedding.

Vaccine challenge studies have been carried out in the USA using a clade 2.3.4.4b North American virus from 2022. One study involved broiler chickens or SPF leghorns and used rHVT vector vaccines with a 2.2 insert and a computationally optimised broadly reactive antigen rHVT vector vaccine. Lee *et al.* (2024) reported good protection following challenge for both vaccines. In another study involving SPF leghorns, used an inactivated rgH5N1

clade 2.3.4.4c vaccine and alphavirus RNA particle vaccine with the HA from a North American H5 clade 2.3.4.4b virus from 2022, both providing 100% protection and reduced viral shedding (Spackman *et al*., 2024).

Studies performed on wild bird clade 2.3.4.4b isolates from 2021 in China demonstrated that the Re-14 vaccine antigen (H5N8 clade 2.3.4.4b virus in a PR8 backbone) was a relevant match for these viruses and provided good protection in ducks (Tian *et al.,* 2023).

Experimental studies may not reflect the field situation, and trials with chickens may not be applicable to other species; however, collectively the above trials demonstrate protection from disease from tested vaccines and reduction in shedding from post 2021, to H5N1 clade 2.3.4.4b viruses.

In some regions, clade 2.3.4.4b viruses coexist with other, antigenically different endemic strains meaning multivalent vaccines may need to be considered to ensure fully protective immunity against HPAI.

Europe

Recent HPAI events in Europe have been described in detail elsewhere (EFSA, 2024). Viruses detected in wild birds and poultry in Europe in 2023 have been antigenically characterised and demonstrated to be antigenically similar. In January 2024, two outbreaks of HPAI A(H5N1) in vaccinated ducks in France were reported to WOAH. These viruses have not been antigenically characterised, however there was no evidence of onwards transmission nor genetic changes associated with vaccine escape. The timing of infection of these ducks was consistent with either early (i.e., not receiving full vaccination course) or waning immunity from vaccination rather than changes to the antigenicity of the virus. Viruses which emerge in vaccinated populations, especially if they contain genetic changes, should be subject to additional characterisation as a matter of importance , as discussed in OFFLU AIM Module 2.

Africa

From available data most viruses detected in poultry in sub-Saharan Africa are derived from introductions of clade 2.3.4.4b from Europe in 2021 however some viruses have been detected in wild birds from subsequent introductions. Clade 2.3.4.4b viruses spreading into Africa have largely replaced other previously circulating clade viruses. In North Africa endemic clade 2.3.4.4b H5N8 continues to co-circulate alongside H5N1.

Antigenic characterization of 18 clade 2.3.4.4b H5N1 viruses, from four sub-Saharan African countries collected between 2021 and 2023 displayed a similar pattern of antigenic reactivity to other Eurasian clade 2.3.4.4b viruses, however these viruses are broadly spread across the 2.3.4.4b antigenic group. Recent (2023) viruses from one African country

exhibited potential antigenic drift possibly after circulating for some time in poultry; however this could not be attributed to any particular amino acid changes. Information on antigenic characteristics of recent (2023) clade 2.3.4.4b viruses from Egypt is not available. Enhanced diagnostic evaluation and timely release of global data is required in order to timely understand vaccine requirements across much of the African continent.

North America

Outbreaks of avian influenza continue to be reported in commercial poultry and backyard flocks in the USA, although at a lower rate compared to last year. The majority of detections in North America are caused by the H5N1 subtype however there have been some reports of H5N5 and H5N6 in wild birds (all clade 2.3.4.4b). There are no reports regarding the antigenic characteristics of these recent viruses using chicken antisera, but viruses appear to have few mutations in known antigenic sites. Less than 1% of viruses detected in dairy cattle have a predicted glycosylation at site 156 (H5 numbering) which, when tested using ferret antisera raised against a 2.3.4.4b clade virus results in a slight change in antigenicity compared to viruses without this change (WHO, 2024b). This loss in reactivity has not been validated using chicken sera and this change has not been observed in sequence data from poultry in North America.

Central and South America

A(H5N1) Gs/Gd clade 2.3.4.4b viruses were first detected in Central and South America in late 2022 and have spread to the southern tip of South America and onward into Antarctica. Outbreaks in this region have had a disproportionate impact on the backyard poultry sector. Some countries have declared freedom from infection in poultry though viruses continue to circulate in wild birds. Viruses from the region were introduced through multiple wild bird movement events in late 2022 and are diverging genetically from viruses detected in North America in 2022 (Jiminez-Bluhm e*t al.,* 2023, Ruiz-Saenz *et al.,* 2023). Recent isolates from poultry are not available from countries where vaccines have been deployed and it is important to continue to monitor viruses, especially in areas where vaccination is being used, to fully characterize them antigenically. Genetically representative isolates from wild birds in the region from 2023 were antigenically characterised and clustered with other clade 2.3.4.4b viruses however some of these viruses demonstrated increasing antigenic distances from early clade 2.3.4.4b viruses. This warrants close monitoring and testing of these viruses as they evolve especially in areas of enzootic circulation in poultry.

Asia

Clade 2.3.4.4b viruses are widespread across much of Asia and many countries have experienced multiple incursions of virus introduced into poultry in 2020 and 2021. Since

October 2023, H5N1 of multiple clades plus different subtypes (H5N5, H5N6 and H5N8) clade 2.3.4.4b viruses have been detected in poultry or wild birds. The clade 2.3.4.4b appears dominant but there is still notable circulation of other clade viruses in some countries.

Clade 2.3.4.4b viruses appears to have replaced other Gs/Gd clades in China and a similar trend has been observed in other countries in Bangladesh and parts of Southeast Asia though not in others. China updated vaccine antigens in early 2022 following the re-introduction of clade 2.3.4.4b viruses (Shi *et al.,* 2023). H5N1, H5N6 and H5N8 clade 2.3.4.4b viruses isolated in China in 2021-2022 exhibited subtype-specific antigenic heterogeneity (Lin *et al.,* 2024) as also reported by Soda *et al.,* (2023) using H5N1 and H5N8 clade 2.3.4.4b viruses isolated in Japan between 2021 and 2022.

Limited information is available on the antigenic characteristics of recent viruses from the region, however OFFLU AIM tested clade 2.3.4.4b viruses from two Asian countries from 2023. The H5N1 viruses tested from South Asia clustered antigenically with other Eurasian H5N1 viruses. The H5N1 viruses tested from Southeast Asia are from various introductions into Southeast Asian poultry but limited genomic surveillance data from this region means it is unclear how representative these viral strains are. These viruses, although clustering with other clade 2.3.4.4b strains were not antigenically homogeneous. This highlights the importance of viral and antigenic monitoring for the early detection of divergent evolution especially in areas of enzootic circulation of virus in poultry. Furthermore, it highlights the importance of selecting strains representative of contemporary viruses if the decision to implement vaccination using an autogenous inactivated vaccine were to be taken.

As reported in the last AIM report, in Indonesia the clade 2.3.4.4b viruses were reported for the first time in 2022 in domestic ducks (Wibawa *et al.,* 2024). These viruses were antigenically characterised against clade 2.3.2.1[1], 2.3.2.1c[1] strains which are used in vaccines in Indonesia. Results suggested that the 2.3.4.4b viruses were antigenically different therefore updating with new vaccine candidates which are antigenically matched to circulating viruses may be required if these new introductions result in spillover to domestic birds. In this scenario it may require the use of bivalent or trivalent H5 seed strains across multiple Gs/Gd clades (OFFLU, 2023).

Clade 2.3.4.4h

Multiple clade 2.3.4.4h viruses were detected in humans in China in 2024 indicating there may still be some circulation of this virus clade in poultry despite the lack of reports and

[1] Smith GJ, Donis RO, World Health Organization, World Organisation for Animal Health, Food Agriculture Organization, H5 Evolution Working Group. Nomenclature updates resulting from the evolution of avian influenza A(H5) virus clades 2.1.3.2a, 2.2.1, and 2.3.4 during 2013-2014. Influenza Other Respir Viruses 2015;9(5):271-6. Available at: http://onlinelibrary.wiley.com/doi/10.1111/irv.12324/epdf.

infrequent detection since 2022, following update of the poultry vaccine, to include a more contemporary 2.3.4.4h antigen. Reports indicate there is genetic divergence of these viruses and accumulation of amino acid changes compared to the 2018 CVV however the significance of these changes is unclear (especially with respect to poultry vaccines), since no antigenic data was available at the time of writing (WHO, 2024c). Furthermore, some of the recent A(H5N6) clade 2.3.4.4h viruses have accumulated over 15 HA amino acid substitutions relative to available CVVs. The extent of the circulation of this clade is unknown. It should be noted that one clade 2.3.4.4h virus from Southeast Asia in 2019 was antigenically characterised in the recent AIM programme and was not recognised by antisera to clade 2.3.4.4b, 2.3.4, 2.2 nor clade 1 viruses and therefore was not included in the antigenic maps. It was antigenically different to clade 2.3.4.4b viruses. Vaccines targeting clade 2.3.4.4 viruses would be unlikely to provide protection against other antigenically different clades.

Clade 2.3.2.1c[1]

Clade "2.3.2.1c[1]" viruses have genetically diversified since their emergence, therefore in new proposed yet unpublished virus nomenclature, those which are currently referred to in-country as clade 2.3.2.1c[1] have been re-designated clade 2.3.2.1e/g/c[2]. It is important to acknowledge that these differently named clades are not related to newly emerging viruses, but rather reflect changes and updated nomenclature due to continuous viral evolution.

One clade 2.3.2.1g[2] virus from the Indonesian Archipelago collected in 2022 was antigenically characterised and was not recognised by clade 2.3.4.4b, 2.3.4, 2.2 nor clade 1 antisera and thus was not included in the antigenic maps. It was antigenically different to clade 2.3.4.4b viruses. Indonesia has a good system in place for updating vaccines when antigenic variants emerge (Hartaningsih *et al.,* 2015). Vaccines targeting clade 2.3.4.4 viruses would be unlikely to provide protection against clade 2.3.2.1c[1] viruses. This clade will be further characterised and addressed by OFFLU in future work.

One clade 2.3.2.1e[2] virus from Southeast Asia collected in 2018 was antigenically characterised and was not recognised by clade 2.3.4.4b, 2.3.4, 2.2 nor clade 1 antisera and thus was not included in the antigenic maps. It was antigenically different to clade 2.3.4.4b viruses. Further studies are needed on the antigenic characteristics of recent clade "2.3.2.1c[1]" viruses from Cambodia, Viet Nam and Laos, however genetic information and antigenic characterisation using ferret antisera suggests these viruses are diversifying

[2] Provisional nomenclature was taken from the WHO/FAO/WOAH H5 Nomenclature Working Group who define "clades" using HA gene sequences, and define clades as genetically distinct, monophyletic groups of viruses. This nomenclature splits clade 2.3.4.4 into eight additional sub-clades, named 2.3.4.4a through 2.3.4.4h due to high circulating diversity within the clade and. well as subclades 2.3.2.1a through 2.3.2.1g for the 2.3.2.1 split.

and may be somewhat antigenically variable, but are mostly recognised by clade 2.3.2.1 sera (WHO, 2024a). Tests should be carried out on these viruses to ensure poultry vaccine match, a priority for OFFLU AIM's next report. Inactivated vaccines based on clade 2.3.4.4b would not be expected to provide consistent protection against clade 2.3.2.1c[1] viruses.

Clade 2.3.2.1a

Viruses of the clade 2.3.2.1a have been circulating since 2011 and continue to be detected in poultry in some countries in South Asia despite incursions of clade 2.3.4.4b viruses into poultry (Islam *et al.*, 2024). Xing *et al.,* (2024) concluded that the clade 2.3.2.1d RE-12 inactivated vaccine provided the best protection against disease in chickens for pre-2019 clade 2.3.2.1 viruses detected in China, however the viruses detected recently in South Asia are considerably genetically divergent from test strains in the study. Dutta *et al.,* (2024) reported the detection of clade 2.3.2.1a in chickens and ducks in asymptomatic vaccinated backyard poultry in Bangladesh in 2017, however Kwon *et al.,* (2021) found vaccines in use (clade 2.3.2.1a and clade 2.2) still offered protection against disease, despite some antigenic variation in clade 2.3.2.1a viruses detected in 2017. In the WHO February 2024 report on the genetic and antigenic characteristics of zoonotic influenza A viruses (WHO 2024a), two antigenic groups of 2.3.2.1a viruses were described, using ferret antisera, defined by the amino acid present at position 154 (H5 numbering). Viruses tested in OFFLU AIM are thought to be genetically representative of contemporary circulating viruses, despite the relatively limited genomic data available. Viruses tested from 2023 demonstrated antigenic variability. Compared to viruses tested from previous years, the antigenic distance between test viruses and antisera raised against surrogate viruses for vaccine seed strains had increased. This indicates that a subset of these viruses circulating in the South Asian region are drifting antigenically and further studies are required to assess vaccine efficacy against contemporary clade 2.3.2.1a viruses to ensure that protection against disease can be provided by vaccines which are currently in use. This should include analysis of sera from field vaccinated birds against a panel of contemporary clade 2.3.2.1a antigens. Consideration should be given for the need to update antigens of conventional inactivated vaccines which are intended to provide protection against clade 2.3.2.1a viruses.

Conventional inactivated vaccines derived from clade 2.3.4.4 viruses would likely not provide appropriate protection against clade 2.3.2.1a viruses based on the large antigenic differences between these clades, therefore when multiple clade viruses (for example clade 2.3.2.1a and clade 2.3.4.4b) are known to be circulating, the use of multi-valent vaccines should be considered.

Conclusions

The major current concern continues to be global outbreaks of clade 2.3.4.4b Gs/Gd HPAI viruses and their circulation in poultry. Present knowledge supports there are no indications of recent significant antigenic variation in viruses of this clade. These viruses have already spread to many countries including some where vaccines are being used to protect against other Gs/Gd clade viruses. It is likely that in the future that region specific antigenic variation will occur as the virus evolves in different ecosystems under different pressures. This will necessitate timely monitoring and characterisation of regional local strains. It should also be noted that in Asia in particular careful monitoring of multiple sub clades of 2.3.2.1 viruses is required as they diversify in avian populations. It is recommended that scientific data informs the need to update vaccines. This may include actively considering inclusion of multiple (multivalent) antigens in vaccines when it is known that several clades are co-circulating or actively threatening poultry populations in a region.

This report provides information on the antigenic characteristics of Gs/Gd A(H5) viruses and on studies, including vaccine challenge trials, that can support decisions when designing or modifying vaccination programs. Other information such as analyses of blood samples taken from vaccinated birds (with known vaccinal history) can provide invaluable insights to vaccine response profiles against contemporaneously circulating viruses in field settings and should also be considered alongside tracking for emergence of virus variants especially but not exclusively in vaccinated populations.

The comprehensive and completeness of the antigenic data in this report depends on the availability of information and sharing of samples from countries experiencing outbreaks of avian influenza. Some data available through pandemic preparedness for humans has identified possible antigenic variation in some groups of viruses and the AIM programme will evaluate these data in the context of poultry, which are known to respond differently to avian influenza antigens compared to ferrets, which are used as a surrogate for humans. Viral isolates and sequences from outbreaks that occur in vaccinated flocks are of great importance and should be prioritised. OFFLU AIM data, a global public good acting with scientific impartiality, are only as strong and effective as the shared information. We strongly encourage national authorities to share this information with OFFLU but also utilise the data generated in the programme to inform their decision making on vaccine strain selection.

We strongly encourage your thoughts on the OFFLU AIM project, and how we can assist you. Please give your feedback via this link.

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