World Health Organization

# Genetic and antigenic characteristics of clade 2.3.4.4b A(H5N1) viruses identified in dairy cattle in the United States of America

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During 2020, highly pathogenic avian influenza A(H5N1) viruses of clade 2.3.4.4b emerged and rapidly spread across many parts of Africa, Asia and Europe. In late 2021, the 2.3.4.4b A(H5N1) viruses were detected in Canada and have since spread across North and South America. In addition to infection of wild and domestic birds, these viruses have been detected in a number of mammals, many presumably infected through exposure to infected birds or contaminated environments. The most recent example of mammalian infection with clade 2.3.4.4b viruses is dairy cattle in the United States. A joint FAO/WHO/WOAH preliminary assessment of recent influenza A(H5N1) viruses including those from cattle has been published[1].

An integral component of WHO's influenza pandemic preparedness activities is the development of candidate vaccine viruses (CVVs) representing influenza viruses of zoonotic and pandemic risk. The genetic and antigenic characteristics of zoonotic influenza A viruses and development of candidate vaccine viruses for pandemic preparedness are summarized and published twice a year[2]. Here we provide an interim assessment of the genetic and antigenic characteristics of clade 2.3.4.4b A(H5N1) viruses isolated from dairy cattle and a dairy farm worker in the United States (US) compared to clade 2.3.4.4b CVVs. Additional whole genome characterization of viruses from dairy cattle and the human case has been described elsewhere[3,4].

A dairy farm worker from the US state of Texas was confirmed infected with a clade 2.3.4.4b A(H5N1) virus similar to those recently found in cattle[4]. The hemagglutinin (HA) of the virus isolated from the worker, A/Texas/37/2024, was closely related to those of the A/Astrakhan/3212/2020 (IDCDC-RG71A) and the A/American wigeon/South Carolina/22-000345-001/2021 (IDCDC-RG78A) CVVs, both of which have been available to vaccine manufacturers since 2022 and 2023, respectively. There were four amino acid changes (L104M, L115Q, T195I, V210A) between the mature HA1 of A/Texas/37/2024 and the A/Astrakhan/3212/2020 CVV and two changes (L115Q, T195I) compared to the A/American wigeon/South Carolina/22-000345-001/2021 CVV (Table 1a and 1b). The changes were not in major antigenic epitopes. Correspondingly, A/Texas/37/2024 was recognized well by post-infection ferret antiserum raised to the A/Astrakhan/3212/2020 and A/American wigeon/South Carolina/22-000345-001/2021 CVVs. Post-infection ferret antiserum raised to an additional clade 2.3.4.4b CVV under development, A/chicken/Ghana/AVL-763_21VIR7050-39/2021-like (IDCDC-RG80A), also recognized well A/Texas/37/2024 well (Table 2a). A/chicken/Ghana/AVL-763_21VIR7050-39/2021-like virus was recommended for the development of a CVV in September 2022[5] when some A(H5N1) viruses from Asia, eastern Europe and West Africa were less well recognized by antisera raised to the A/Astrakhan/3212/2020 CVV. Viruses isolated from infected dairy cattle also reacted well with at least one of the available clade 2.3.4.4b CVVs (Table 2b and 2c). Genetic analysis of 225 clade 2.3.4.4b cattle virus sequences made publicly available by the US Department of Agriculture identified the same HA changes as those found in the human case from Texas in 100% of cattle viruses. An HA amino acid change resulting in a predicted gain of glycosylation (A156T) in antigenic site B was detected in a virus isolated from a cow in Texas that was tested by HI (Table 2b). When tested by ferret antisera raised to IDCDC-RG71A and IDCDC-RG78A, these antisera had reduced reactivity to this virus likely as a result of the glycosylation. In contrast, this virus was inhibited well by ferret antisera raised to IDCDC-RG80A, which also contains the HA A156T change. To date, this change has been detected in only 4% of cattle viruses. Additional HA changes relative to the clade 2.3.4.4b CVVs were detected, but at low frequency among viruses identified in cattle

[1] https://www.who.int/publications/m/item/joint-fao-who-woah-preliminary-assessment-of-recent-influenza-a(h5n1)-viruses
[2] https://www.who.int/teams/global-influenza-programme/vaccines/who-recommendations
[3] https://www.biorxiv.org/content/10.1101/2024.05.01.591751v1
[4] https://www.nejm.org/doi/full/10.1056/NEJMc2405371
[5] https://cdn.who.int/media/docs/default-source/influenza/who-influenza-recommendations/vcm-southern-hemisphere-recommendation-2023/202209_zoonotic_vaccinvirusupdate.pdf

and, to date, do not appear to represent changes that have spread among cattle (Table 1a). Nonetheless, continued antigenic characterization of viruses will be important to inform future pandemic preparedness and evaluation of CVVs.

**Based on current genetic, antigenic and epidemiologic data, no new CVVs are proposed**.

Selection and development of CVVs are the first steps towards timely vaccine production and do not imply a recommendation for initiating manufacture. National authorities may consider the use of one or more of these CVVs for pilot lot vaccine production, clinical trials and other pandemic preparedness purposes based on their assessment of public health risk and need.

Currently, clinical studies of several vaccines manufactured from A(H5) CVVs are underway to evaluate the immune response in humans and to assess cross-reactive antibody titers of sera from these trials when tested with viruses associated with outbreaks in cattle. These data will support the decisions of national authorities to license and approve A(H5) vaccines for use in humans if necessary.

**Table 1a**. Hemagglutinin changes detected in U.S. dairy cattle outbreak viruses compared to the candidate vaccine virus, IDCDC-RG71A (A/Astrakhan/3212/2020-like)

| Residue | IDCDC-RG71A | B3.13* | Total Count | Frequency Detected in B3.13 cattle viruses (%) |
|---|---|---|---|---|
| 97 | D | E | 1 | 0.44% |
| 104 | L | M | 225 | 100% |
| 115 | L | Q | 225 | 100% |
| 127 | T | A | 21 | 9.33% |
| 156 | A | T | 9 | 4.00% |
| 195 | T | I | 225 | 100% |
| 210 | V | A | 225 | 100% |
| 236 | D | Y | 1 | 0.44% |
| 272 | G | S | 1 | 0.44% |
| 285 | I | M | 1 | 0.44% |
| 320 | S | N | 18 | 8.00% |
| 321 | P | L | 4 | 1.78% |
| 336 | I | I/K | 1 | 0.44% |
| 356 | H | L | 1 | 0.44% |
| 407 | I | V | 1 | 0.44% |
| 452 | V | A | 1 | 0.44% |
| 494 | E | G | 4 | 1.78% |
| 511 | I | V | 225 | 100% |
| 523 | A | V | 1 | 0.44% |
| 528 | A | V | 1 | 0.44% |
| 531 | I | S | 6 | 2.67% |

* Genotype B3.13 is the nomenclature used to describe the reassortant genotype of viruses identified in dairy cattle and the dairy farm worker[6].

[6] Virology. 2023 Oct:587:109860. https://doi.org/10.1016/j.virol.2023.109860.

**Table 1b**. Hemagglutinin changes detected in U.S. dairy cattle outbreak viruses compared to the candidate vaccine virus, IDCDC-RG78A (A/American wigeon/South Carolina/22-000345-001/2021-like)

| Residue | IDCDC-RG78A | B3.13 | Total Count | Frequency Detected in B3.13 cattle viruses (%) |
|---|---|---|---|---|
| 97 | D | E | 1 | 0.44% |
| 115 | L | Q | 225 | 100% |
| 127 | T | A | 21 | 9.33% |
| 156 | A | T | 9 | 4.00% |
| 195 | T | I | 225 | 100% |
| 236 | D | Y | 1 | 0.44% |
| 272 | G | S | 1 | 0.44% |
| 285 | I | M | 1 | 0.44% |
| 320 | S | N | 18 | 8.00% |
| 321 | P | L | 4 | 1.78% |
| 336 | I | I/K | 1 | 0.44% |
| 356 | H | L | 1 | 0.44% |
| 407 | I | V | 1 | 0.44% |
| 452 | V | A | 1 | 0.44% |
| 494 | E | G | 4 | 1.78% |
| 523 | A | V | 1 | 0.44% |
| 528 | A | V | 1 | 0.44% |
| 531 | I | S | 6 | 2.67% |

**Table 2a**. Hemagglutination inhibition assay of HPAI A(H5Nx) viruses

| **REFERENCE ANTIGENS**[7,8] | **Subtype** | **Clade** | **IDCDC-RG71A** | **IDCDC-RG78A** | **IDCDC-RG80A** |
|---|---|---|---|---|---|
| IDCDC-RG71A (A/Astrakhan/3212/2020-like) | H5N8 | 2.3.4.4b | **160** | 80 | 160 |
| IDCDC-RG78A (A/American Wigeon/South Carolina/22-000345-001/2021-like) | H5N1 | 2.3.4.4b | 80 | **160** | 320 |
| IDCDC-RG80A (A/chicken/Ghana/AVL-763_21VIR7050-39/2021-like) | H5N1 | 2.3.4.4b | 40 | 40 | **320** |
| **TEST ANTIGENS** | | | | | |
| A/Texas/37/2024, conjunctival swab isolate | H5N1 | 2.3.4.4b | 80 | 160 | 320 |
| A/Texas/37/2024, nasopharyngeal swab isolate | H5N1 | 2.3.4.4b | 40 | 160 | 320 |

**Table 2b**. Hemagglutination inhibition assay of HPAI A(H5Nx) viruses

| **REFERENCE ANTIGENS** | **Subtype** | **Clade** | **IDCDC-RG71A** | **IDCDC-RG78A** | **IDCDC-RG80A** |
|---|---|---|---|---|---|
| IDCDC-RG71A (A/Astrakhan/3212/2020-like) | H5N8 | 2.3.4.4b | **320** | 40 | 2560 |
| IDCDC-RG78A (A/American Wigeon/South Carolina/22-000345-001/2021-like) | H5N1 | 2.3.4.4b | 80 | **160** | 2560 |
| IDCDC-RG80A (A/chicken/Ghana/AVL-763_21VIR7050-39/2021-like) | H5N1 | 2.3.4.4b | 40 | 40 | **2560** |
| **TEST ANTIGENS** | | | | | |
| A/bovine/Texas/75952/2024 | H5N1 | 2.3.4.4b | 160 | 80 | 2560 |
| A/bovine/Texas/97794/2024 (HA A156T; gain of glycosylation) | H5N1 | 2.3.4.4b | 80 | 40 | 2560 |

**Table 2c**. Hemagglutination inhibition assay of HPAI A(H5Nx) viruses

| **REFERENCE ANTIGENS** | **Subtype** | **Clade** | **CBER-RG8A** | **AmWigeon/2021** |
|---|---|---|---|---|
| CBER-RG8A (A/Astrakhan/3212/2020-like) | H5N8 | 2.3.4.4b | **80** | 40 |
| A/American wigeon/South Carolina/22-000345-001/21 | H5N1 | 2.3.4.4b | 80 | **160** |
| **TEST ANTIGENS** | | | | |
| A/bovine/Ohio/B24OSU-432/2024 | H5N1 | 2.3.4.4b | 80 | 40 |
| A/bovine/Ohio/B24OSU-413/2024 | H5N1 | 2.3.4.4b | 80 | 80 |

[7] https://cdn.who.int/media/docs/default-source/influenza/cvvs/cvv-zoonotic-northern-hemisphere-2024-2025/h5n1_summary_a_h5n1_cvv_20240223.pdf
[8] https://cdn.who.int/media/docs/default-source/influenza/cvvs/cvv-zoonotic-northern-hemipshere-2023-2024/h5-non-h5n1_cvv_-20231003_20240223.pdf

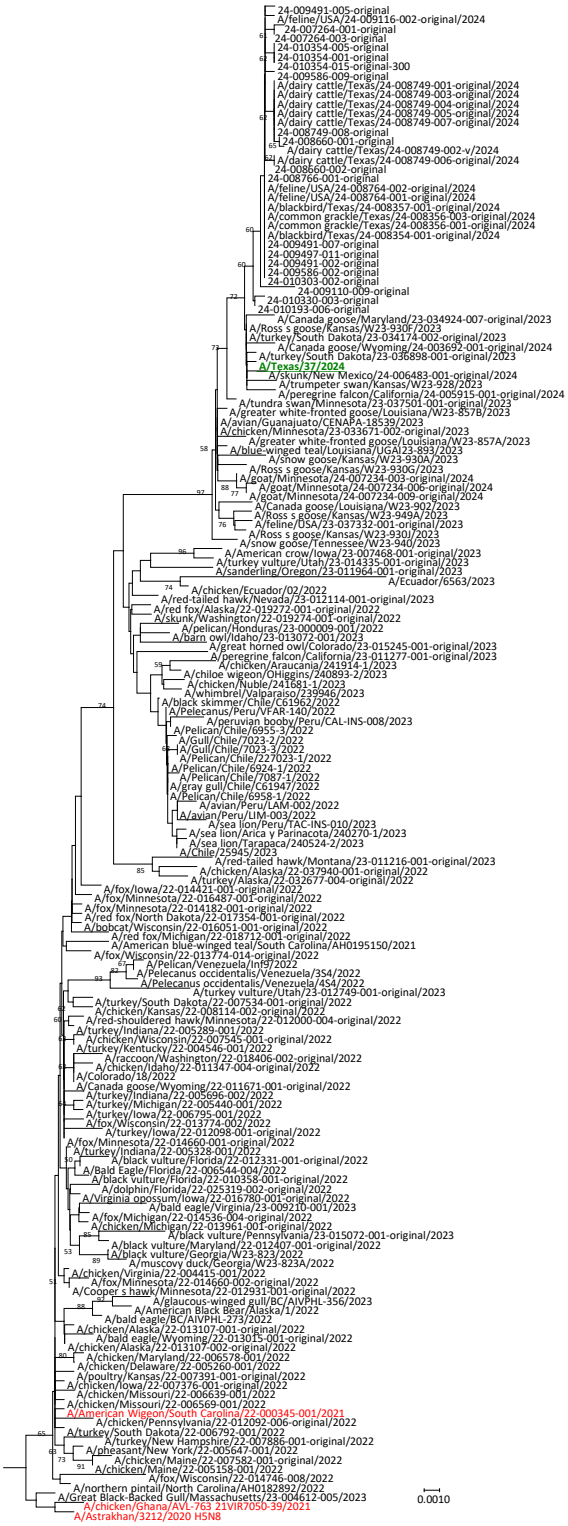


**Figure 1.** Phylogenetic relationships of influenza A(H5) HA genes of clade 2.3.4.4b. CVVs that are available or in preparation are in red. Human case is in green.