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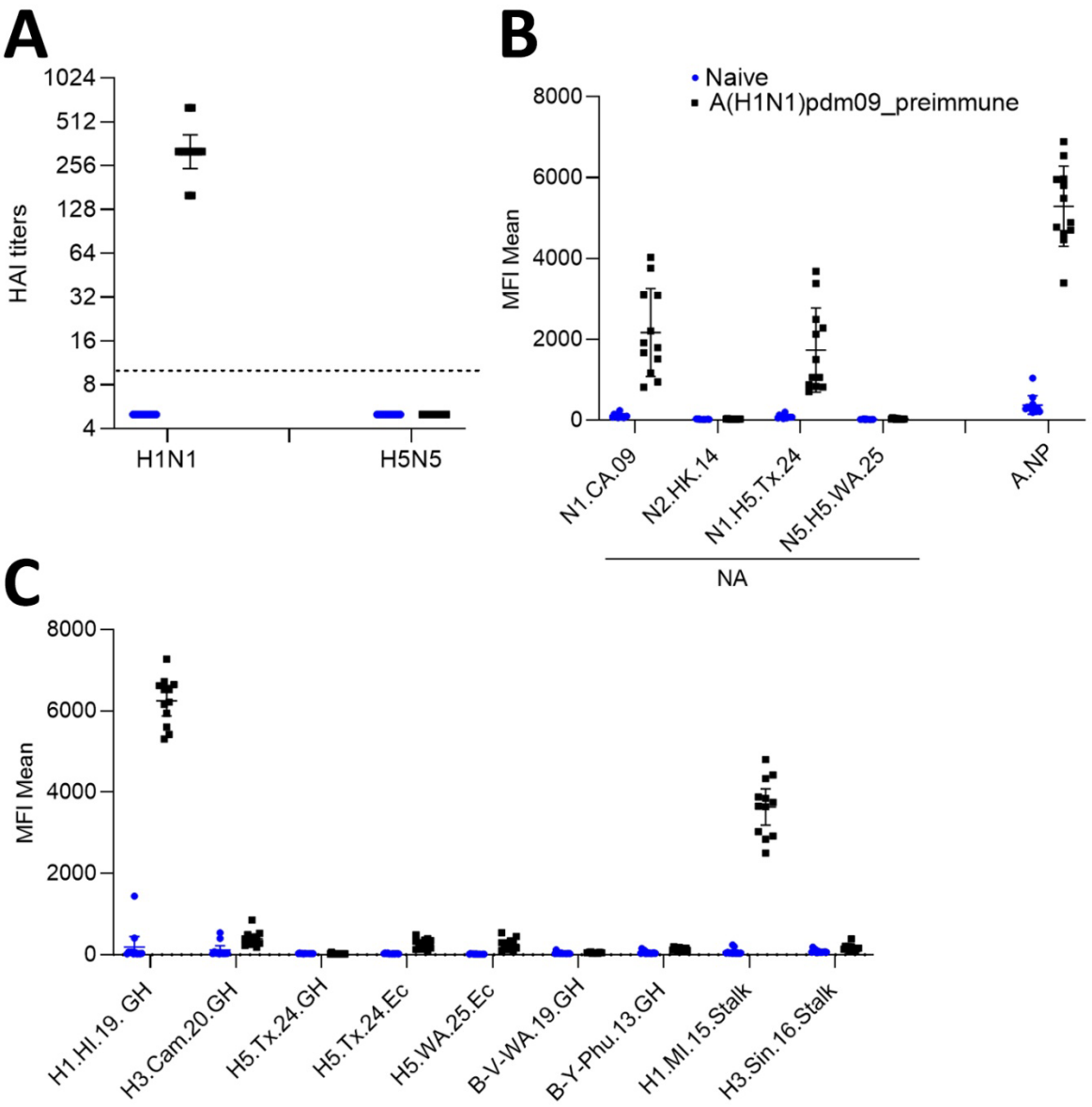
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Cross-Protection against Influenza A(H5) Clade 2.3.4.4b Viruses after A(H1N1)pdm09 Infection

Appendix

Appendix Reference

1. Yassine HM, Boyington JC, McTamney PM, Wei CJ, Kanekiyo M, Kong WP, et al. Hemagglutinin-stem nanoparticles generate heterosubtypic influenza protection. Nat Med. 2015;21:1065–70. PubMed https://doi.org/10.1038/nm.3927



Appendix Figure. Detection of HAI antibodies and binding antibodies after A(H1N1)pdm09 infection in ferrets. We intranasally inoculated 12 serologically naive ferrets with $10^{3.0}$ PFU of NE/14 virus. Serum samples were collected before inoculation and at 90 days after inoculation (A(H1N1)pdm09_preimmune). We subsequently evaluated sera for HAI (A). Scatter dot plots with solid lines represent the geometric means with 95% CIs. The limit of detection indicated by the dashed line in the HAI assay is 10, and any sample with an HAI titer <10 was assigned to a titer of 5. We tested serum for binding antibodies by using a multiplex influenza binding antibody detection assay (MIADA) against influenza A virus NA and NP (B), and HA GH, Ec, and stalk, including part of HA1 and HA2 defined previously (*1*) (C). Scatter dot plots with solid lines represent the mean median fluorescence intensity values with 95% CIs for MIADA assays. NP proteins were derived from A(H3N2) A/Brisbane/10/2007 virus. The HA proteins used in the MIADA included B-V-WA.19.GH, influenza B Victoria lineage B/Washington/02/2019 GH; B-Y-Phu.13.GH,

influenza B Yamagata lineage B/Phuket/3073/2013 GH; H1.HI.19.GH, A (H1N1)pdm09 A/Hawaii/70/2019 GH; H1.MI.15.Stalk, A(H1N1)pdm09 A/Michigan/45/2015 HA stalk; H3.Cam.20.GH, A(H3N2) A/Cambodia/e0826360/2020 GH; H3.Sin.16.Stalk, A(H3N2) A/Singapore/INFIMH-16–0019/2016 HA stalk; H5.Tx.24.Ec, A(H5N1) A/Texas/37/2024 Ec; H5.Tx.24.GH, A(H5N1) A/Texas/37/2024 GH; H5.WA.25.Ec, A(H5N5) A/Washington/2148/2025 Ec. The NA antigens included N1.CA.09, A(H1N1) A/California/07/2009; N1.H5.Tx.24, A(H5N1) A/Texas/37/2024; N2.HK.14, A(H3N2) A/Hong Kong/4108/2014; N5.H5.WA.25, A(H5N5) A/Washington/2148/2025. Ec, ectodomain; GH, globular head; HA, hemagglutinin; HAI, hemagglutinin inhibition; MFI, mean fluorescence intensity; NA, neuraminidase; NE/14, A/Nebraska/14/2019; NP, nucleoprotein.