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

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We challenged ferrets previously infected with influenza A(H1N1)pdm09 virus with H5N1 or H5N5 clade 2.3.4.4b viruses to assess cross-protection. Results show that previous A(H1N1)pdm09 virus immunity conferred partial protection against both viruses, with slightly reduced protection against H5N5 compared with H5N1, potentially reflecting the absence of cross-reactive N5 neuraminidase antibodies.

Influenza A(H5) clade 2.3.4.4b viruses have spread widely among wild birds and poultry and caused infections in mammals, including humans, raising public health concerns (*1*). A case of human infection with H5N5 clade 2.3.4.4b virus was reported in Washington, USA during 2025; the patient subsequently died, representing the second fatal A(H5) case in the United States (*1*,*2*). A(H1) and A(H5) hemagglutinins (HAs) belong to influenza A virus phylogenetic group 1. Most antibodies targeting the HA stalk, a conserved, membrane-proximal region of HA, are cross-reactive with HAs within the same phylogenetic group (*3*). Studies have demonstrated that previous influenza A(H1N1)pdm09 virus infection can provide some protection against H5N1 clade 2.3.4.4b viruses (*4*,*5*). The emergence of H5N5 clade 2.3.4.4b virus raises questions about whether previous A(H1N1)pdm09 immunity confers similar protection against A(H5) viruses bearing antigenically distinct neuraminidases (NAs). We used a preimmune ferret model to compare cross-protection conferred by previous A(H1N1)pdm09 infection against A(H5) viruses with homologous or heterologous NA subtypes. We conducted all experiments with H5N5 and H5N1 viruses in a Biosafety Level 3 enhanced facility with approval from the Centers for Disease Control and Prevention Institutional Animal Care and Use Committee.

We intranasally inoculated 12 ferrets serologically naive to seasonal influenza viruses with 10^3 PFU of A(H1N1)pdm09 A/Nebraska/14/2019 virus. We confirmed seroconversion 3 months postinfection by using a hemagglutination inhibition (HAI) assay, which detects antibodies that inhibit HA-mediated hemagglutination, with a geometric mean titer of 320 (95% CI 245.3–417.3) (Appendix Figure, panel A; https://wwnc.cdc.gov/EID/article/32/12/26-1032-App1.pdf). Using previously described multiplex binding assays (*6*), we detected high levels of antibodies binding to the head of A(H1N1)pdm09, but not HA head or ectodomain from H5N1 or H5N5 viruses, and antibodies binding to N1 NAs from A(H1N1)pdm09 and H5N1 clade 2.3.4.4b viruses, but not N5 NA from H5N5 virus. In addition, we detected antibodies binding to the group 1 HA stalk derived

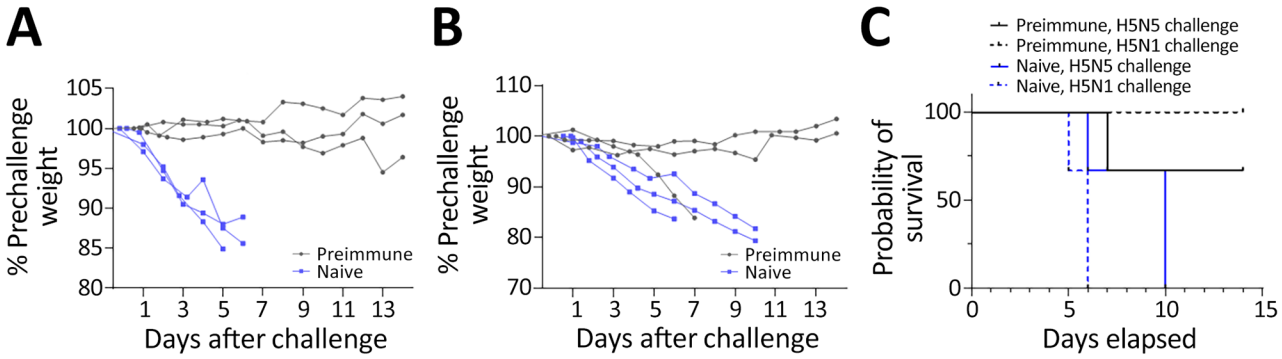


Figure 1. Pathogenicity of influenza A(H5N1) and A(H5N5) clade 2.3.4.4b viruses from study of cross-protection after A(H1N1)pdm09 infection. We challenged ferrets previously intranasally inoculated with A/Nebraska/14/2019 (H1N1) virus (A[H1N1]pdm09 (preimmune group) 3 months later with 10^4 PFU of A/Washington/239/2024 (H5N1) virus or A/Washington/2148/2025 (H5N5) virus (n = 6 per virus group; gray), along with naive control ferrets (n = 6 per virus group; blue). We monitored 3 ferrets per group daily for clinical signs of disease and weight loss. Percentage bodyweight relative to prechallenge after challenge with H5N1 (A) or H5N5 (B) baseline for individual ferrets. C) Kaplan-Meier survival curves; survival difference between 2 preimmune groups assessed by log-rank (Mantel-Cox) test was not significant (p>0.05).

from A(H1N1)pdm09 virus (Appendix Figure, panels B, C). We then intranasally challenged those animals, referred to as preimmune ferrets, with 10^4 PFU of H5N1 clade 2.3.4.4b A/Washington/239/2024 or H5N5 A/Washington/2148/2025 virus (6 ferrets per virus), alongside naive control groups without previous influenza immunity. We euthanized 3 ferrets per group on day 4 postinfection to assess viral dissemination; we monitored the remaining animals for clinical signs, weight loss, viral shedding in nasal washes, and virus presence in throat and rectal swabs.

Severe disease, characterized by lethargy, fever, and substantial weight loss (maximum 13.8% in H5N1 virus inoculated ferrets and 18.4% in H5N5

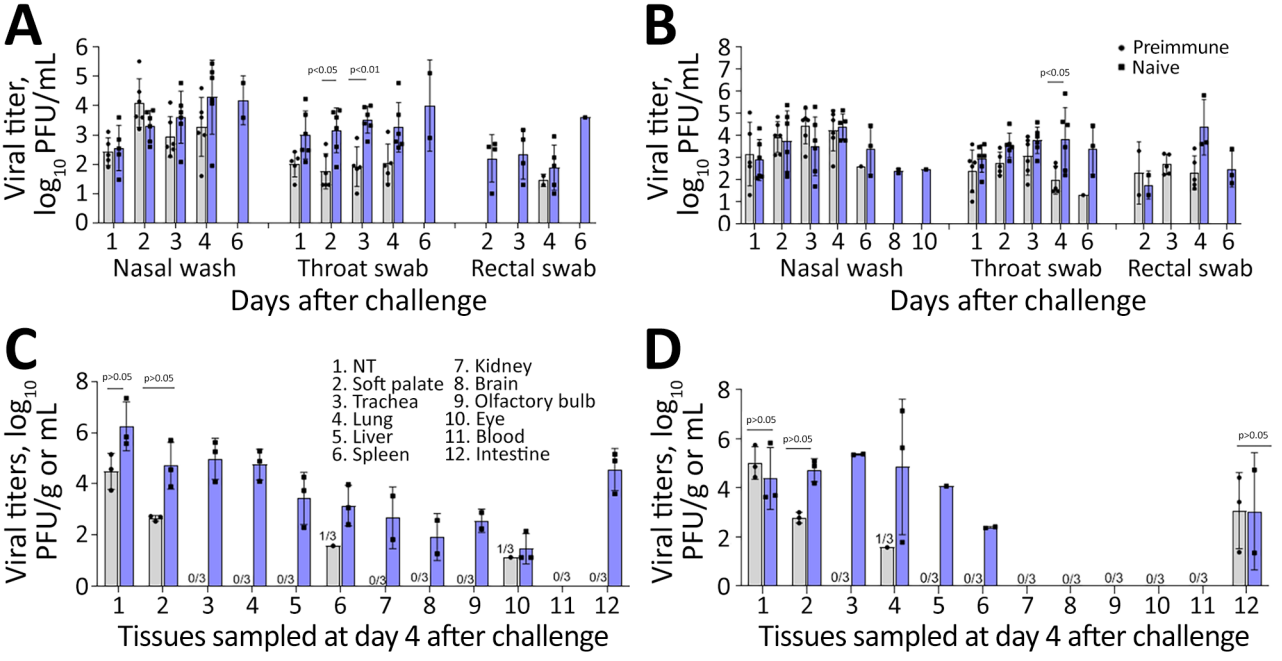


Figure 2. Viral titers after A(H5N1) and A(H5N5) challenge in ferrets in a study on cross-protection against influenza A(H5) clade 2.3.4.4b viruses after A(H1N1)pdm09 infection. A, B) Mean viral titers in nasal washes and throat and rectal swab specimens after challenge with H5N1 (A) or H5N5 (B) virus. We assessed statistical differences between preimmune and naive groups at each time point by using 2-way analysis of variance with Sidak's multiple comparisons test after confirming normality with Shapiro-Wilk test. C, D) Mean viral titers in tissue and blood specimens ($\log_{10}$ PFU/g for tissues or mL for blood) on day 4 postinfection with either H5N1 (C) or H5N5 (D) virus. Symbols indicate individual preimmune or naive animals. Error bars represent SDs. When ≤3 ferrets had detectable virus, the number positive/3 necropsied ferrets is indicated. When all 3 ferrets in preimmune and naive groups had detectable virus, we assessed differences by using the Mann-Whitney test. NT, nasal turbinates.

virus inoculated ferrets), developed in naive ferrets inoculated with H5N1 or H5N5 viruses. We euthanized all animals on postinfection days 5–6 for H5N1 virus–inoculated ferrets and days 6–10 for H5N5 virus–inoculated ferrets after they reached predefined humane endpoints, consistent with previous reports (*7*,*8*). In contrast, preimmune ferrets challenged with H5N1 virus exhibited minimal disease, a mean maximum weight loss of 2.4%, and 100% survival (Figure 1, panels A, C). Preimmune ferrets challenged with H5N5 also showed reduced disease severity (mean maximum weight loss 7.6%); however, 1 animal reached a humane endpoint on day 7 postinfection (Figure 1, panels B, C).

Preimmune ferrets challenged with either H5N1 or H5N5 virus shed similar levels of virus in nasal wash samples during days 1–4 postinfection (range of mean peak titers $10^{3.8}$–$10^{5.2}$ PFU/mL) as naive controls but exhibited reduced titers in throat swab samples on day 4 for H5N5 virus and days 2 and 3 for H5N1 virus (Figure 2, panels A, B). Viral clearance from nasal washes occurred 2–4 days earlier in preimmune ferrets. We detected H5N1 virus less frequently in rectal swab samples from preimmune animals than from naive controls, whereas we did not observe that reduction for ferrets infected with H5N5 virus (Figure 2, panels A, B).

Influenza A(H1N1)pdm09 virus preimmunity did not substantially reduce viral titers in the upper respiratory tract (nasal turbinates) or soft palate after H5N1 or H5N5 virus challenge (Figure 2, panels C, D). However, A(H1N1)pdm09 preimmunity largely prevented viral dissemination to the lower respiratory tract (trachea and lungs) and extrapulmonary tissues for both viruses, with only sporadic low-level viral detection. However, intestinal viral titers were comparable between preimmune and naive ferrets challenged with H5N5 virus (Figure 2, panels C, D).

Detection of group 1 HA stalk antibodies after A(H1N1)pdm09 infection (Appendix Figure), together with previous evidence of HA stalk-mediated cross-protection against disease caused by the influenza A viruses within the same phylogenetic group (*3*,*9*), suggests that cross-reactive HA stalk antibodies might contribute to protection against A(H5) viruses. In addition, N1 cross-reactivity might further enhance protection against H5N1 virus. Consistent with our findings, limited N5 cross-reactivity has been reported in humans despite detectable N1 responses (*10*). Further studies using H5-specific HA stalk antigens and functional assays are needed to determine the contribution of cross-reactive antibodies and cellular immunity to protection against zoonotic influenza A viruses. Our findings showed that previous A(H1N1)pdm09 viral infection conferred partial cross-protection against H5N1 and H5N5 viruses in ferrets, reducing disease severity, lethality, and viral dissemination.

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Natural *Trichuris trichiura* Infections in Endemic Hydromyini Rodents, Papua New Guinea, 2019–2023

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Screening of 874 wild mammals in Papua New Guinea during 2019–2023 identified *Trichuris* whipworm infections in multiple rodent species. Mitochondrial and nuclear markers identified 8 *Trichuris trichiura* infections. Those findings broaden the recognized host range of this human soil-transmitted helminth and raise questions regarding wildlife involvement in its transmission ecology.

[1]These senior authors contributed equally to this article.

The *Trichuris trichiura* whipworm is a soil-transmitted helminth that infects ≈500 million persons worldwide (*1*). Despite environmental transmission through infective eggs, *T. trichiura* is generally considered to be maintained exclusively by humans and nonhuman primates (*2*). Although whipworms infect a wide range of mammalian hosts, including rodents (*3*,*4*) and marsupials (*5*), *Trichuris* species whipworms generally are considered host-specific, and experimental studies show that *T. trichiura* does not readily establish patent infections in rodents or other nonprimate hosts (*1*; K.A. Stark et al., unpub. data, https://doi.org/10.1101/2025.05.09.653168). All major soil-transmitted helminths, including *T. trichiura*, are endemic in Papua New Guinea (*7*; J.L. Scott, master's thesis, James Cook University, 2023). We investigated *Trichuris* whipworm infections in wild mammals in Papua New Guinea and unexpectedly identified *T. trichiura* in multiple endemic rodents.

We sampled mammals during 2019–2023 at 19 localities across 2 mountain systems and 3 lowland sites (Appendix 1 Figure 1, https://wwwnc.cdc.gov/EID/article/32/12/26-1101-App1.pdf). We examined the cecum and colon of 874 animals for whipworms (Appendix 1; Appendix 2 Table, https://wwwnc.cdc.gov/EID/article/32/12/26-1101-App2.xlsx). The Ethical Committee of the Faculty of Science, University of South Bohemia approved the animal research protocol (approval no. 2019-1).

Rodents in Papua New Guinea come from 2 major murine lineages: Rattini, which includes endemic and introduced *Rattus* species, and Hydromyini, an endemic Australo–Papuan adaptive radiation occupying diverse ecologic niches. Of the 874 tested animals, 43 marsupials tested negative for whipworms, although the small sample distributed across multiple taxa precludes inference regarding their susceptibility. Sixteen rodents harbored *Trichuris* spp. whipworms: 7 *Rattus niobe* rats and 9 Hydromyini rodents representing 7 species (Table). The 7 infections in *R. niobe* rats occurred at a single location, whereas infections in Hydromyini rodents were distributed among several locations and host lineages.

Because morphologic species delimitation within *Trichuris* is difficult, we genetically characterized worms from 11 infected rodents (Appendix 1). The mitochondrial genomes of 8 specimens shared 97.5%–97.7% nucleotide identity with a human-derived *T. trichiura* reference sequence from Ecuador (GenBank accession no. AP017704) (Appendix 1 Table 2). Each