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Experimental H5N1 mRNA Vaccine Cuts Avian Influenza Viral Shedding in Dairy Cow Milk by 1,000-Fold

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Since the March 2024 identification of highly pathogenic avian influenza (HPAI) H5N1 clade 2.3.4.4b (genotype B3.13) in U.S. dairy herds, the virus has challenged a long-standing paradigm of influenza virology . Rather than spreading primarily as an upper-respiratory infection via airborne droplets, bovine-adapted H5N1 exhibits pronounced tropism for the alveolar secretory epithelium of the lactating bovine mammary gland , replicating to exceptionally high titers in raw milk and driving cow-to-cow transmission across milking parlors. Now, in a landmark challenge study published in Nature Biotechnology [1] , immunologists at the University of Pennsylvania Perelman School of Medicine and veterinary virologists at the U.S. Department of Agriculture Agricultural Research Service (USDA-ARS) National Animal Disease Center report that an experimental nucleoside-modified mRNA- lipid nanoparticle (mRNA-LNP) vaccine reduces peak H5N1 viral RNA shedding in the milk of lactating dairy cows by approximately 1,000-fold (3 log 10 units) while protecting mammary tissue from necrotizing mastitis and fibrosis [1,2] .

Why Clade 2.3.4.4b H5N1 Targets the Bovine Mammary Gland

To understand why a parenterally administered vaccine must establish immunity inside the udder, it is necessary to examine the receptor biology of influenza A viruses. The viral surface glycoprotein hemagglutinin (HA) initiates host-cell entry by binding to terminal sialic acid (N -acetylneuraminic acid) residues attached to galactose on host cell-surface glycans. Whereas human-adapted seasonal influenza viruses (such as H1N1 and H3N2) preferentially bind alpha2,6-linked sialic acids abundant in the human upper respiratory tract, avian-origin H5N1 viruses preferentially bind alpha2,3-linked sialic acids (specifically alpha2,3-sialyl-Galbeta1,4-GlcNAc motifs) [4,5] .

Histochemical mapping of cattle tissues following the initial 2024 outbreak revealed that while the bovine upper respiratory tract expresses relatively modest densities of avian-type receptors, the secretory luminal epithelial cells lining the mammary alveoli and lactiferous ducts of lactating dairy cows express dense arrays of alpha2,3-linked sialic acids [4,5] . When trace volumes of contaminated milk are mechanically introduced through the teat canal via milking-claw liners during commercial parlor operations, H5N1 clade 2.3.4.4b infects the alveolar epithelium directly. In unvaccinated cows, viral replication inside the udder reaches infectious titers of 10 6 to 10 8 TCID 50 mL 1 (and quantitative RT-PCR cycle thresholds below 15), lysing milk-secreting acinar cells, thickening milk into colostrum-like yellowish exudates, and causing an abrupt 50% to 90% collapse in daily milk yield accompanied by interstitial mammary fibrosis [1,4,5] .

Design of the Penn-USDA Bovine H5 mRNA-LNP Trial

Led by Jefferson J. S. Santos , Scott E. Hensley , and Nobel laureate Drew Weissman at the University of Pennsylvania in partnership with Kaitlyn M. Sarlo Davila and Amy L. Vincent Baker at the USDA-ARS National Animal Disease Center in Ames, Iowa, the research team engineered a lipid nanoparticle-encapsulated mRNA vaccine encoding the full-length hemagglutinin of a contemporary clade 2.3.4.4b H5N1 isolate [1,2] . As in human mRNA vaccines, uridine residues in the mRNA transcript were replaced with N 1 -methylpseudouridine (m 1) to prevent premature innate immune degradation by endosomal Toll-like receptors (TLR7 and TLR8) and maximize translational output inside antigen-presenting cells [1] .

Following preliminary dose-ranging and immunogenicity validation in mice and Holstein calves [2] , the investigators conducted a high-containment biosafety level 3 agriculture (BSL-3 Ag) challenge trial in lactating Holstein dairy cows . Cattle received a two-dose intramuscular prime-boost vaccination regimen, while control cows remained unvaccinated. Four months after the primary immunization series, both cohorts were challenged directly via the intramammary route -- inoculating a high dose of live bovine clade 2.3.4.4b H5N1 virus directly into the teat cisterns to simulate worst-case parlor cross-contamination [1] .

| Experimental Parameter / Endpoint | | Unvaccinated Control Lactating Cows | | H5 mRNA-LNP Vaccinated Lactating Cows (Prime-Boost) | | Epidemiological & Clinical Significance |

| Pre-Challenge Serum & Milk H5 Antibody Titers | | Undetectable baseline H5-specific IgG and microneutralization titers | | High H5-binding IgG and neutralizing antibody titers in both systemic serum and secreted milk | | Systemic intramuscular immunization successfully transudates neutralizing IgG across the blood-milk barrier |

| Peak Viral RNA Shedding in Milk (Post-Challenge) | | High-titer shedding sustained across 7-14 days post-inoculation | | ~1,000-fold (~3-log 10) reduction in peak viral genomic RNA concentration in milk | | Suppresses infectious shedding toward or below the threshold required for milking-machine transmission |

| Clinical Mastitis & Daily Milk Production | | Acute clinical mastitis, abnormal clotted milk, fever, and severe drop in daily milk yield | | Preserved normal milk appearance and sustained daily lactation volume with minimal systemic febrile response | | Protects dairy herd productivity and prevents premature culling of high-producing cows |

| Mammary Histopathology at Necropsy | | Widespread alveolar epithelial necrosis, leukocytic infiltration, and interlobular mammary fibrosis | | Intact secretory acinar architecture with markedly reduced inflammation and absence of severe fibrosis | | Prevents permanent loss of functional quarter secretory capacity across subsequent lactations |

| Vaccine-Derived mRNA Residue in Milk | | Not applicable | | Undetectable (0 copies above limit of detection) in serial post-vaccination milk samples | | Confirms that intramuscular mRNA-LNP remains localized to the injection site and draining lymph nodes |

Transudation of Neutralizing Antibodies Across the Blood-Milk Barrier

A central immunological question prior to the trial was whether a vaccine injected intramuscularly into the neck or gluteal musculature of a 650-kilogram cow could protect the mucosal lumen of the mammary gland against direct teat-canal inoculation. In ruminants , unlike humans (where dimeric secretory IgA predominates in breast milk), the dominant immunoglobulin isotype transported across the mammary alveolar epithelium into colostrum and mature milk is immunoglobulin G1 (IgG1) , which is actively shuttled from maternal serum across the basolateral-to-apical membrane of lactocytes by the neonatal Fc receptor (FcRn) [1,2] .

Serial sampling in the Penn-USDA trial confirmed that the second (booster) dose of the H5 mRNA-LNP vaccine triggered a robust germinal-center response, elevating H5 hemagglutinin-specific neutralizing antibodies not only in peripheral blood serum, but also directly inside secreted milk [1] . When live H5N1 virus was infused into the teat canal four months later, pre-existing milk-borne neutralizing antibodies and rapidly recruited memory B and T lymphocytes neutralized viral particles before extensive cell-to-cell spread could occur across the mammary parenchyma. As a result, peak viral RNA loads in the milk of vaccinated cows were reduced by roughly 1,000-fold compared to unvaccinated controls, and infectious virus clearance was markedly accelerated [1] .

Food Safety Verification and One Health Spillover Prevention

Because commercial adoption of an mRNA livestock vaccine requires rigorous consumer and regulatory confidence in dairy food safety, the investigators performed sensitive quantitative reverse- transcription PCR (RT-qPCR) assays on milk collected from vaccinated cows immediately following immunization. Across all time points examined, no vaccine-derived mRNA was detectable in milk [1] , confirming that the lipid nanoparticles remain confined to the intramuscular injection depot and draining regional lymph nodes.

From a One Health perspective, curbing viral replication in the mammary gland addresses three converging biosecurity threats:

* Breaking Intra-Herd and Inter-Herd Transmission Chains: Mechanical transfer of high-titer milk on milking units and movement of subclinically shedding lactating cattle have driven H5N1 dissemination across more than 1,000 U.S. dairy herds since 2024 [4,5] . Reducing peak milk viral loads by three orders of magnitude substantially lowers the environmental infectious dose inside milking parlors.

* Protecting Calves and Barnyard Peridomestic Species: Companion USDA-ARS research published in Nature Communications [3] confirmed that calves fed unpasteurized H5N1-contaminated waste milk develop respiratory and systemic infection, while raw milk spills have repeatedly infected domestic cats, peridomestic wild birds, and poultry flocks.

* Reducing Mammalian Evolutionary Opportunity and Farm-Worker Exposure: Every high-titer replication cycle inside a bovine host provides opportunities for the viral RNA-dependent RNA polymerase (specifically the PB2 subunit at residues E627K, D701N, and M631L) to select for mammalian polymerase adaptations and potential

reassortment with human seasonal influenza strains in exposed dairy workers [4,5] .

While deployment of any commercial H5N1 cattle vaccine in the United States remains subject to USDA Center for Veterinary Biologics licensure, trade-surveillance protocols (such as Differentiating Infected from Vaccinated Animals, or DIVA serology using nucleoprotein-specific ELISAs alongside hemagglutinin-only mRNA antigens), and cost-per-dose scaling, the Penn-USDA challenge data establish that an HA-only mRNA-LNP platform can rapidly match evolving clade 2.3.4.4b genotypes and shield the bovine mammary gland from high-titer viral amplification [1,2] .

References & Primary Sources

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