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The risk of transmission of influenza A H5N1 virus through direct contact with raw milk from infected dairy cows (the mammary gland of cows abundantly displays receptors for circulating 2.3.4.4b H5 viruses) | 1

Influenza A viruses H5N1 clade 2.3.4.4b are circulating in dairy farms in the United States. The transmission route to mammals is under debate. Recent studies have investigated the ongoing risk of transmission of highly pathogenic avian influenza (HPAI) A(H5N1) virus to humans through direct contact with raw milk from infected dairy cows, the binding of 2.3.4.4b H5 influenza A viruses to available receptors in the mammary gland and upper and lower respiratory tract tissue samples from cows, whether untreated milk positive for HPAI A (H5N1) poses a risk of infecting susceptible animals that consume it, and the stability of HPAI A(H5N1) viruses in cow's milk stored at 4°C.

A recent case report described an adult dairy farmworker who experienced discomfort in his right eye one day after milk splashed in that eye while he was milking a cow at a farm that had confirmed HPAI A(H5N1) infection in dairy cows. He had not been using personal protective equipment. A day after symptom onset, an examination of the right eye showed mildly erythematous conjunctiva, consistent with conjunctivitis. There was nothing notable about the left eye. A reverse transcription polymerase chain reaction (RT-PCR) testing of a conjunctival swab from the right eye and a nasopharyngeal swab taken a day after symptom onset demonstrated that the conjunctival specimen was positive for Influenza A(H5), while the nasopharyngeal swab was negative. The virus from the conjunctival swab was later identified as HPAI A (H5N1). (Morse J. et al. Influenza A(H5N1) Virus Infection in Two Dairy Farm Workers in Michigan. *N Engl J Med* 2024;391:963-964)

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Another recent study investigated whether 2.3.4.4b H5 influenza A viruses can bind to available receptors in formalin-fixed and paraffin-embedded samples of the upper (nasal or trachea) and lower (lung) respiratory tract and mammary gland tissues from cows as well as of respiratory tract tissues from horses and pigs. These animals were not previously infected with the HPAI A (H5N1) virus. The findings demonstrated that cow mammary glands abundantly display receptors for currently circulating 2.3.4.4b H5 viruses. The classical H5 protein, H5IN showed binding to lactating cows but not to non-lactating cows. However, the upper respiratory tract of cows did not display influenza A virus receptors. H5VN Y161A mutant was the only H5 protein significantly bound to the cow upper respiratory tract, but, its binding receptor is not a receptor for circulating 2.3.4.4b H5N1 viruses. A similar pattern was observed in horse and pig trachea. In contrast, almost all hemagglutinin proteins, including human H1PR8, bound to cow lung tissue with varying intensity, demonstrating that receptors are present in the lungs. (Carrasco MR et al. The mammary glands of cows abundantly display receptors for circulating avian H5 viruses. *J of Virology*, 10 October 2024) <https://journals.asm.org/doi/10.1128/jvi.01052-24>

Another animal study investigated whether untreated milk positive for HPAI A (H5N1) poses a risk for susceptible animals that consume it. A day after the oral inoculation of NM#93 milk sample positive for HPAI A (H5N1) to BALB/cJ mice, the animals showed signs of illness, including ruffled fur and lethargy. All animals survived until day four when they were euthanized to determine virus titers in multiple organs. High virus titers were detected in the respiratory organs and moderate titers in several other organs, consistent with systemic infections caused by HPAI H5 viruses. Importantly, the virus was detected in the mammary glands of two mice. This is consistent with the findings of a high viral load in the milk of lactating cows, even though these mice were not lactating, and the above-mentioned findings of abundantly displayed receptors for 2.3.4.4b H5 viruses in the mammary glands of cows.

In the same study, the researchers explored heat inactivation of milk samples positive for HPAI A(H5N1) viruses. The inoculation of undiluted milk samples positive for HPAI A (H5N1) viruses (previously incubated in a PCR thermocycler at 63°C for 5, 10, 20, or 30 minutes or at 72°C for 5, 10, 15, 20, or 30 seconds) into embryonated chicken eggs or canine kidney cells showed that heat treatment for 15 or 20 seconds did not completely inactivate the virus. However, the authors asserted that bench-top experiments do not



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recapitulate commercial pasteurization processes. The HPAI A (H5N1) virus stability assessment in cow's milk stored at 4°C revealed a decline of only two log units over 5 weeks. According to the authors, the HPAI A(H5N1) virus could remain infectious for several weeks in raw milk stored at 4°C. (Guan L, Eisfeld AJ, Pattinson D, et al. Cow's Milk Containing Avian Influenza A(H5N1) Virus -Heat Inactivation and Infectivity in Mice. N Engl J Med 2024;391:87-90) <https://www.nejm.org/doi/full/10.1056/NEJMc2405495>

Another study which also investigated the heat treatment of H5N1 HPAIV-positive milk samples using three pasteurization methods: 63°C for 30 minutes, 72°C for 15 seconds, or 80°C for 15 seconds confirmed these results. Only pasteurization at 80°C for 15 seconds completely inactivated the viruses present in milk. (Hu W. et al. The Thermal Stability of Influenza Viruses in Milk. Viruses 2024, 16(11), 1766) <https://www.mdpi.com/1999-4915/16/11/1766>

