2. Batista-Dos-Santos SA, Freitas DRC, Raiol M, Cabral GF, Feio AC, Póvoa MM, et al. Strategy to improve malaria surveillance system preventing transfusion-transmitted malaria in blood banks using molecular diagnostic. Malar J. 2018;17:344. https://doi.org/10.1186/s12936-018-2486-z
3. Schwartz BS, Mawhorter SD; AST Infectious Diseases Community of Practice. Parasitic infections in solid organ transplantation. Am J Transplant. 2013;13:280–303. https://doi.org/10.1111/ajt.12120
4. Instituto de Salud Carlos III. ISCIII portal web [cited 2024 Nov 3]. https://www.isciii.es
5. European Centre for Disease Prevention and Control. Annual epidemiological report for 2022: malaria [cited 2024 Dec 26]. https://www.ecdc.europa.eu/en/publications-data/annual-epidemiological-report-2022-malaria
6. ISCIII-Vigilancia en Salud Pública-RENAVE. ISCIII portal web [cited 2024 Nov 3]. https://www.isciii.es/servicios/vigilancia-salud-publica-renave
7. Ministerio de Sanidad. Informes de situación y evaluación del riesgo publicados por el CCAES [cited 2024 Nov 3]. https://www.sanidad.gob.es/areas/alertasEmergenciasSanitarias/situacionRiesgo/informes.htm
8. National Institute of Allergy and Infectious Diseases (NIAID). Malaria [cited 2024 Nov 3]. https://www.niaid.nih.gov/diseases-conditions/malaria
9. Bottius E, Guanzirolli A, Trape JF, Rogier C, Konate L, Druilhe P. Malaria: even more chronic in nature than previously thought: evidence for subpatent parasitaemia detectable by the polymerase chain reaction. Trans R Soc Trop Med Hyg. 1996;90:15–9. https://doi.org/10.1016/S0035-9203(96)90463-0
10. Aguas R, Maude RJ, Gomes MGM, White LJ, White NJ, Dondorp AM. Infectivity of chronic malaria infections and its consequences for control and elimination. Clin Infect Dis. 2018;67:295–302. https://doi.org/10.1093/cid/ciy055

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Avian Influenza A(H5N1) Isolated from Dairy Farm Worker, Michigan, USA

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Influenza A(H5N1) viruses have been detected in US dairy cow herds since 2024. We assessed the pathogenesis, transmission, and airborne release of A/Michigan/90/2024, an H5N1 isolate from a dairy farm worker in Michigan, in the ferret model. Results show this virus caused airborne transmission with moderate pathogenicity, including limited extrapulmonary spread, without lethality.

Highly pathogenic avian influenza A(H5N1) clade 2.3.4.4b viruses have displayed unprecedented global spread among wild birds leading to numerous spillover infections in mammalian species. Of note, outbreaks in dairy cattle and gallinaceous birds have resulted in human infections in the United States during 2024–2025 (*1*). Increased frequency of H5N1 viruses crossing species barriers has caused concern that the avian influenza viruses are adapting to mammals. A critical component of influenza pandemic preparedness is early identification of emerging novel influenza viruses that cause disease and transmit efficiently in humans. A clade 2.3.4.4b H5N1 virus, A/Michigan/90/2024 (MI90), genotype B3.13, was isolated from a conjunctival swab specimen collected from a human patient in Michigan with conjunctivitis after exposure to infected cattle (*2*,*3*). In this article, we report the pathogenesis, transmission, and airborne exhalation of MI90 virus in ferrets, the standard animal model for influenza virus risk assessments (*4*).

We inoculated 18 ferrets with MI90 virus as previously described (*5*,*6*). We euthanized 3 ferrets on 3 and 5 days postinoculation (dpi) to assess virus spread in tissues. We used 6 ferrets to assess transmission in a cohoused, direct contact setting as a direct contact transmission model and through the air in the absence of direct or indirect contact as a respiratory droplet transmission model. We paired each ferret with a naive contact, as previously described (*4*). We observed clinical manifestations daily and collected

Table. Clinical signs and virus titers in ferrets infected with avian influenza A(H5N1) isolated from dairy farm worker in Michigan, 2024*

Clinical signs and tissues sampled	Inoculated ferrets		Transmission models		
	Euthanized at 3 dpi	Euthanized at 5 dpi	Inoculated	DCT	RDT
Weight loss, %†	4.5 (3/3)	11.8 (3/3)	9.8 (12/12)	5.5 (6/6)	6.6 (3/6)
Fever, °C above baseline‡	0.9 (3/3)	1.3 (2/3)	1.8 (11/12)	1.8 (6/6)	2.0 (3/6)
Nasal wash	6.1 (3/3)	5.4 (3/3)	5.1 (1–5 d)	4.6 (5–7 d)	4.5 (9–11 d)
Conjunctival wash§	1.4 (3/3)	NT	3.2 (3 d)	ND	ND
Rectal swab¶	1.4 (3/3)	NT	2.6 (3–5 d)	1.0 (3 d)	1.4 (3 d)
Tissues					
Nasal turbinate	6.6 (3/3)	5.3 (3/3)	NT	NT	NT
Ethmoid turbinate	7.4 (3/3)	6.5 (3/3)	NT	NT	NT
Soft palate	3.5 (1/3)	NT	NT	NT	NT
Lung#	3.5 (2/3)	4.3 (3/3)	NT	NT	NT
Trachea#	5.9 (3/3)	5.8 (2/3)	NT	NT	NT
Intestine#	1.8 (1/3)	ND (0/3)	NT	NT	NT
Brain#	2.4 (3/3)	2.4 (2/3)	NT	NT	NT
Olfactory bulb	3.1 (2/3)	4.2 (3/3)	NT	NT	NT

*Values are $\log_{10}$ PFU/mL (no. ferrets affected/total no. in group) except as indicated. DCT, direct contact transmission model; ND, not detected; NT, not tested; RDT, respiratory droplet transmission model.
†Mean maximum weight loss after inoculation with 10^6 PFU A/Michigan/90/2024 A(H5N1) virus in a 1-mL volume.
‡Mean maximum rise in body temperature from baseline (37.4°C–39.0°C).
§Conjunctival washes collected from 6 of 12 inoculated animals and 3 each of DCT and RDT contact ferrets in the transmission experiment; number of ferrets with detectable virus or day of mean peak shown parenthetically.
¶Virus in rectal swab samples detected in 8 of 12 inoculated and 1 each of DCT and RDT contact ferrets in the transmission experiment; number of ferrets with detectable virus or day of mean peak shown parenthetically.
#Values are $\log_{10}$ PFU/g.

nasal wash (NW), conjunctival, and rectal swab samples every 2 days postinoculation or postcontact. We confirmed transmission by testing for seroconversion to homologous virus in the contact animals.

Although all MI90-infected ferrets survived the 21-day study, we noted moderate disease. In inoculated ferrets, the mean maximum weight loss was 9.8%, fever (1.8°C above baseline) and lethargy were transient, and nasal and ocular discharge and sneezing were evident at 4–9 dpi (Table). We detected virus 3 dpi primarily in respiratory tract tissues; titers were highest in ethmoid turbinate samples (7.4 $\log_{10}$ PFU/mL) and at low levels in brain and gastrointestinal tissues. We observed similar results in tissues collected 5 dpi.

During the direct contact transmission experiment, inoculated ferrets shed virus in NW that peaked at 4.7–5.4 $\log_{10}$ PFU/mL at 1–5 dpi (Figure, panel A). Four of 6 cohoused contact animals had virus in NW (peak 2.5–4.9 $\log_{10}$ PFU/mL) at 5–7 days postcontact, whereas all 6 contact animals had viral RNA detected (3.6–7.7 $\log_{10}$ copies/mL) in NW (*7*) and seroconverted to MI90 virus, indicating that transmission was 100% (6/6 animals). In the respiratory droplet transmission experiment, NW collected from inoculated animals peaked 2.6–4.8 $\log_{10}$

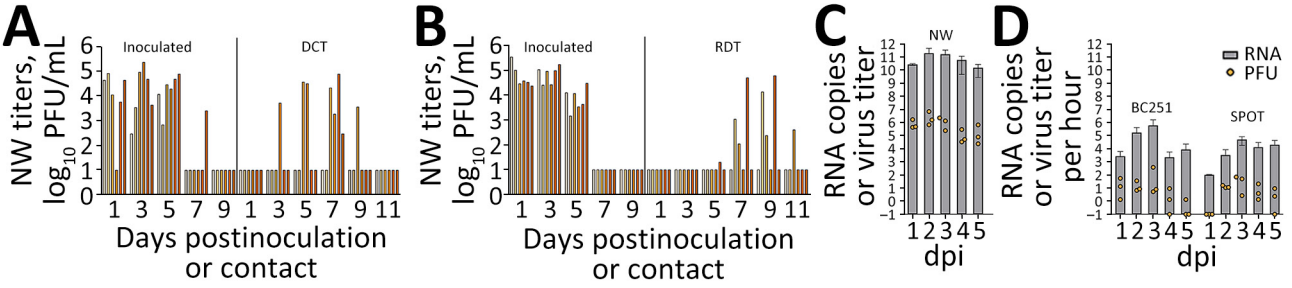

Figure. Transmission and measurement of airborne avian influenza A(H5N1) virus isolated from dairy farm worker, Michigan. A, B) For DCT and RDT testing, ferrets (n = 12) were intranasally inoculated with 10^6 PFU A/Michigan/90/2024 virus, isolated from the dairy worker, in 1 mL phosphate-buffered saline and were cohoused with naive ferrets in a DCT model (A) or in adjacent cages with perforated sidewalls permitting airborne virus spread but restricting contact in an RDT model (B). Each bar represents a single animal. C, D) For aerosol testing, ferrets (n = 3) were inoculated intranasally with 10^6 PFU of MI90 virus and tested daily (C). Orange dots represent viral titers from NW in log10 PFU/mL; limit of detection 10 PFU/mL. Gray bars show average viral M gene RNA load. Error bars indicate SD. Limit of detection was 2.9 log10 RNA copies/mL. D) Aerosol samples were collected daily for 5 dpi by using a BC251 cyclone-based sampler (kindly provided by Dr. William Lindsley, National Institute for Occupational Safety and Health) and the SPOT water condensation sampler (Aerosol Devices, https://aerosoldevices.com), as described previously (8). Orange dots represent log10 PFU/mL per hour. Gray bars show average viral M gene RNA. Error bars indicate SD. Limit of detection was 2.5 log10 RNA copies/h. Ferrets were used for tissue collection on day 5. DCT, direct contact transmission; dpi, days postinoculation; NW, nasal washes; RDT, respiratory droplet transmission.

PFU/mL at 1–3 dpi, whereas 3/6 contact ferrets had detectable virus in NW by day 7 postcontact (peak 2.6–4.8 $\log_{10}$ PFU/mL; days 9–11 postcontact) (Figure, panel B) as well as viral RNA (6.7–8.2 $\log_{10}$ copies/mL), and seroconverted, confirming transmission through the air in 50% of ferrets (3/6). We also detected infectious virus in conjunctival and rectal samples from inoculated animals, but only from 2 contact animals (Table).

To further evaluate the level of virus exhaled by MI90-inoculated ferrets and the potential for airborne transmission, we collected aerosol samples 1 time each day at 1–5 dpi for 1 hour from the 3 ferrets that were euthanized at 5 dpi. Air samples were analyzed for infectious virus and viral RNA by using the BC251 cyclone-based sampler (kindly provided by Dr. William Lindsley, National Institute for Occupational Safety and Health) and the SPOT water condensation sampler (Aerosol Devices, https://aerosoldevices.com), as described previously (*8*) (Figure, panel D). The highest mean titer of virus was detected at 2 dpi in NW collected from all 3 inoculated ferrets (6.5 $\log_{10}$ PFU/mL) (Figure, panel C). Airborne virus was highest at 3 dpi as measured in both samplers, up to 133 and 41 PFU/hour, supporting transmission observed in both contact models within 3–5 days after exposure.

Overall, MI90 virus displayed reduced virulence in ferrets compared with another H5N1 virus isolated from a dairy farm worker in Texas (*8*,*9*); the Texas virus possesses a genetic marker in the polymerase basic 2 protein (E627K), known for enhanced replication and pathogenesis in mammals. At this position, MI90 encodes 627E, like most other viruses isolated from cattle, and contains polymerase basic 2 M631L, which is associated with mammal adaptation (*3*,*9*). In addition, polymerase acidic 142N/E has been linked to increased virulence in mice (*10*); the Texas virus has an E and MI90 virus has a K at this position. Both viruses have identical hemagglutinin sequences associated with receptor binding and the multi-basic cleavage site. Despite differences in virulence, both viruses transmitted in the ferret model with similar proficiency and levels of airborne virus.

Because avian H5N1 viruses cross the species barrier and adapt to dairy cattle, each associated human infection presents further opportunity for mammal adaption. This potential poses an ongoing threat to public health and requires continual surveillance and risk assessment of emerging viruses to improve our ability to predict and prepare for the next influenza pandemic.

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All animal procedures were approved by the Institutional Animal Care and Use Committee of the Centers for Disease Control and Prevention and were conducted in an AAALAC-accredited facility.

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References

1. Webby RJ, Uyeki TM. An update on highly pathogenic avian influenza A(H5N1) virus, clade 2.3.4.4b. J Infect Dis. 2024;230:533–42. https://doi.org/10.1093/infdis/jiae379
2. Garg S, Reed C, Davis CT, Uyeki TM, Behravesh CB, Kniss K, et al. Outbreak of highly pathogenic avian influenza a(h5n1) viruses in U.S. dairy cattle and detection of two human cases—United States, 2024. MMWR Morb Mortal Wkly Rep. 2024;73:501–5. https://doi.org/10.15585/mmwr.mm7321e1
3. Morse J, Coyle J, Mikesell L, Stoddard B, Eckel S, Weinberg M, et al. Influenza A(H5N1) virus infection in two dairy farm workers in Michigan. N Engl J Med. 2024;391:963–4. https://doi.org/10.1056/NEJMc2407264
4. Maines TR, Chen LM, Matsuoka Y, Chen H, Rowe T, Ortin J, et al. Lack of transmission of H5N1 avian-human reassortant influenza viruses in a ferret model. Proc Natl Acad Sci U S A. 2006;103:12121–6. https://doi.org/10.1073/pnas.0605134103
5. Maines TR, Lu XH, Erb SM, Edwards L, Guarner J, Greer PW, et al. Avian influenza (H5N1) viruses isolated from humans in Asia in 2004 exhibit increased virulence in mammals. J Virol. 2005;79:11788–800. https://doi.org/10.1128/JVI.79.18.11788-11800.2005
6. Belser JA, Gustin KM, Maines TR, Pantin-Jackwood MJ, Katz JM, Tumpey TM. Influenza virus respiratory infection and transmission following ocular inoculation in ferrets. PLoS Pathog. 2012;8:e1002569. https://doi.org/10.1371/journal.ppat.1002569
7. Pulit-Penaloza JA, Brock N, Belser JA, Sun X, Pappas C, Tumpey TM, et al. Kinetics and magnitude of viral RNA shedding as indicators for influenza A virus transmissibility in ferrets. Commun Biol. 2023;6:90. https://doi.org/10.1038/s42003-023-04459-0

8. Pulit-Penaloza JA, Belser JA, Brock N, Kieran TJ, Sun X, Pappas C, et al. Transmission of a human isolate of clade 2.3.4.4b A(H5N1) virus in ferrets. Nature. 2024;636:705–10. https://doi.org/10.1038/s41586-024-08246-7
9. Gu C, Maemura T, Guan L, Eisfeld AJ, Biswas A, Kiso M, et al. A human isolate of bovine H5N1 is transmissible and lethal in animal models. Nature. 2024;636:711–8. https://doi.org/10.1038/s41586-024-08254-7
10. Kim JH, Hatta M, Watanabe S, Neumann G, Watanabe T, Kawaoka Y. Role of host-specific amino acids in the pathogenicity of avian H5N1 influenza viruses in mice. J Gen Virol. 2010;91:1284–9. https://doi.org/10.1099/vir.0.018143-0

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Aedes aegypti Mosquito Detection at Bus Stations, Bogota, Colombia, 2023–2024

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We monitored mosquitoes in 3 bus stations in Bogota, Colombia, located at 2,625 m above sea level. During December 2023–January 2024, we collected 27 larvae and 1 adult female *Aedes aegypti* mosquitoes at 1 station. Detection of *Ae. aegypti* mosquitoes in Bogota is a call to continue monitoring mosquitoes at stations.

Aedes spp. mosquitoes can feed on many species, including humans (*1*,*2*). *Ae. aegypti* mosquitoes are a public health concern because they can transmit pathogens that cause some of the most common arboviral diseases, such as dengue fever, Zika, chikungunya, and yellow fever (*2*–*4*). Among the *Aedes* mosquito species, *Ae. aegypti* is the most widely studied because of its broad distribution range and widespread association with arboviral transmission, especially dengue virus (*2*,*4*). *Ae. aegypti* mosquitoes are found in tropical climates where temperatures range from 15°C to 30°C, and the altitude is generally ≤1,700 meters above sea level (masl). Some countries, such as Mexico, Peru, and Bolivia, have reported *Ae. aegypti* mosquitoes at >2,000 masl (*1*,*4*–*6*). However, some reports in Colombia note *Ae. aegypti* mosquitoes at altitudes as high as 2,100 masl (*7*,*8*). *Ae. aegypti* mosquitoes are found in most urban and peri-urban areas of Colombia, according to a survey published by the National Health Institute (*7*).

In Colombia, dengue fever is the most common arbovirus disease. In 2024, the country registered 27,649 cases: 15,926 (57.6%) persons showed mild symptoms, 11,419 (41.3%) showed moderate symptoms, and 304 (1.1%) had severe symptoms (*8*). A group of 10 states, Valle del Cauca, Cali, Tolima, Huila, Santander, Norte de Santander, Antioquia, Bolívar, Cundinamarca, and Meta, had 21,392 (77.4%) of those cases. Cundinamarca, the state in which Bogota is located, had 867 reports. Only 36 cases of Zika virus infection were recorded in Colombia in 2024, 12 (33.3%) of which occurred in Cundinamarca. Furthermore, 15 cases of chikungunya virus were documented; of those, 1 (6.6%) case was reported in Cundinamarca in the area around Bogota (*8*). Of note, Bogota is the only place in Cundinamarca with no reports of arboviruses, but notifications have been made in most neighboring municipalities at lower altitudes (200–1,700 masl). Bogota is at 2,600 masl and is considered outside the distribution range of the vectors.

Climate change has increased global temperatures, leading to new arboviral outbreaks. Recent studies have shown that *Ae. aegypti* mosquitoes now inhabit areas that were once outside their distribution range (*1*,*2*,*5*,*6*,*8*). The temperature in Bogota has consistently risen since the 1990s. In the mid-1960s, the average temperature per year was 12.6°C. In 2022, the average temperature reached 13.8°C; the highest temperature recorded was 25.1°C (*9*). That temperature increase suggests that Bogota may no longer be outside the distribution range of *Aedes* spp. mosquitoes. Herein, we report detection of *Ae. aegypti* mosquitoes in the city of Bogota, Colombia.

The possibility of an expansion in the distribution range of *Ae. aegypti* mosquitoes created the need for weekly monitoring and sample collection by the Secretaría Distrital de Salud (https://www.saludcapital.gov.co) of Bogota beginning in May 2023. The sampling efforts focused on the 3 bus stations of the city