

References

1. Waites KB, Talkington DF. *Mycoplasma pneumoniae* and its role as a human pathogen. *Clin Microbiol Rev*. 2004;17:697-728. <https://doi.org/10.1128/CMR.17.4.697-728.2004>
2. Meyer Sauter PM, Beeton ML; European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Study Group for *Mycoplasma* and *Chlamydia* Infections (ESGMAC), and the ESGMAC *Mycoplasma pneumoniae* Surveillance (MAPS) Study Group. *Mycoplasma pneumoniae*: delayed re-emergence after COVID-19 pandemic restrictions. *Lancet Microbe*. 2024;5:e100-e101.
3. Nordholm AC, Søborg B, Jokelainen P, Lauenborg Møller K, Flink Sørensen L, Grove Krause T, et al. *Mycoplasma pneumoniae* epidemic in Denmark, October to December, 2023. *Euro Surveill*. 2024;29:2300707. <https://doi.org/10.2807/1560-7917.ES.2024.29.2.2300707>
4. Morel AS, Dubourg G, Prudent E, Edouard S, Gouriet F, Casalta JP, et al. Complementarity between targeted real-time specific PCR and conventional broad-range 16S rDNA PCR in the syndrome-driven diagnosis of infectious diseases. *Eur J Clin Microbiol Infect Dis*. 2015;34:561-70. <https://doi.org/10.1007/s10096-014-2263-z>
5. Li H, Li S, Yang H, Chen Z, Zhou Z. Resurgence of *Mycoplasma pneumoniae* by macrolide-resistant epidemic clones in China. *Lancet Microbe*. 2024;S2666-5247(23)00405-6.
6. Edens C, Clopper BR, DeVies J, Benitez A, McKeever ER, Johns D, et al. Notes from the field: reemergence of *Mycoplasma pneumoniae* infections in children and adolescents after the COVID-19 pandemic, United States, 2018-2024. *MMWR Morb Mortal Wkly Rep*. 2024;73:149-51. <https://doi.org/10.15585/mmwr.mm7307a3>
7. Zayet S, Poloni S, Plantin J, Hamani A, Meckert Y, Lavoignet CE, et al. Outbreak of *Mycoplasma pneumoniae* pneumonia in hospitalized patients: who is concerned? Nord Franche-Comté Hospital, France, 2023-2024. *Epidemiol Infect*. 2024;152:e46. <https://doi.org/10.1017/S0950268824000281>
8. Diaz MH, Cross KE, Benitez AJ, Hicks LA, Kuty P, Bramley AM, et al. Identification of bacterial and viral codetections with *Mycoplasma pneumoniae* using the TaqMan array card in patients hospitalized with community-acquired pneumonia. *Open Forum Infect Dis*. 2016;3:ofw071. <https://doi.org/10.1093/ofid/ofw071>
9. Koenen MH, de Groot RCA, de Steenhuijsen P, WAA, Chu MLJN, Arp K, Hasrat R, et al. *Mycoplasma pneumoniae* carriage in children with recurrent respiratory tract infections is associated with a less diverse and altered microbiota. *EBioMedicine*. 2023;98:104868. <https://doi.org/10.1016/j.ebiom.2023.104868>
10. Kim K, Jung S, Kim M, Park S, Yang HJ, Lee E. Global trends in the proportion of macrolide-resistant *Mycoplasma pneumoniae* infections: a systematic review and meta-analysis. *JAMA Netw Open*. 2022;5:e2220949. <https://doi.org/10.1001/jamanetworkopen.2022.20949>

Address for correspondence: Florence Fenollar, I
HU-Méditerranée Infection, 19-21 boulevard Jean Moulin, 13005
Marseille, France; email: florence.fenollar@univ-amu.fr

Fatal Infection in Ferrets after Ocular Inoculation with Highly Pathogenic Avian Influenza A(H5N1) Virus

Jessica A. Belser, Xiangjie Sun,
Joanna A. Pulit-Penalzo, Taronna R. Maines

Author affiliation: Centers for Disease Control and Prevention,
Atlanta, Georgia, USA

DOI: <https://doi.org/10.3201/eid3007.240520>

Ocular inoculation of a clade 2.3.4.4b highly pathogenic avian influenza A(H5N1) virus caused severe and fatal infection in ferrets. Virus was transmitted to ferrets in direct contact. The results highlight the potential capacity of these viruses to cause human disease after either respiratory or ocular exposure.

In recent years, clade 2.3.4.4b highly pathogenic avian influenza A(H5N1) viruses have exhibited substantial host expansion, geographic spread, and reassortment with other circulating influenza A viruses (IAVs) in birds, resulting in epornitics on all continents and virus detection in an expanding group of mammals (1). Human cases of H5N1 clade 2.3.4.4b virus infection have been reported, typically following direct exposure to infected animals, contaminated environments, or both (2). A 2.3.4.4b highly pathogenic avian influenza A(H5N1) virus was isolated from a human patient in Chile during 2023 (A/Chile/25945/2023 [Chile/25945]) (3) and caused severe and fatal disease in ferrets intranasally inoculated with 10^6 PFU of virus. Transmission of virus to animals housed in close contact was also reported (3), highlighting the pandemic potential of clade 2.3.4.4b viruses.

Although the eyes represent a secondary mucosal surface that is susceptible to respiratory virus exposure (4), as evidenced by recent reports of conjunctivitis in 2 dairy workers exposed to clade 2.3.4.4b H5N1 virus (5), risk assessment approaches for clade 2.3.4.4b H5N1 viruses to date have been limited to standard intranasal inoculation (3,6) and have not evaluated the capacity of those viruses to cause disease after alternative portals of entry. To investigate relative similarities between ocular and respiratory exposure to H5N1 virus, we assessed the severity and kinetics of disease after ocular exposure of ferrets to Chile/25945 virus and compared our findings with a previously published assessment of animals intranasally inoculated with

this virus at the Centers for Disease Control and Prevention in 2023 (3).

To assess disease severity and transmissibility under different exposure concentrations, we inoculated ferrets by the ocular route with either a high (10^6 PFU) or low (10^3 PFU) dose of Chile/25945 virus (7). At either challenge dose, all ferrets inoculated by the ocular route became productively infected, reaching mean maximum weight loss of 12.7% (high dose) and 13.2% (low dose) and mean maximum rises in temperature of 2.4°C (high dose) and 2.0°C (low dose). Humane endpoints were reached on postinoculation days 5–7 in 3/3 (high dose) and 2/3 (low dose) animals (Figure 1, panel A). During necropsy, we detected infectious virus throughout the respiratory tract and in several extrapulmonary tissues (including from the gastrointestinal tract, central nervous system, and ocular system) (Figure 1, panel C), consistent with the highly virulent phenotype observed after high-dose intranasal inoculation of ferrets (3).

One ferret survived the challenge with a serologic titer of 160.

To assess if ferrets inoculated by the ocular route were as capable as intranasally inoculated ferrets to transmit Chile/25945 virus in a direct contact setting (3), we cohoused a serologically naive ferret with each inoculated ferret 24 hours after inoculation. To assess virus replication within and beyond the respiratory tract, we collected nasal wash, conjunctival wash/swab, and rectal swab samples from inoculated and contact animals. All ferrets inoculated by the ocular route with a high dose of virus had detectable infectious virus in nasal wash (mean peak titer $5.3 \pm 0.2 \log_{10}$ PFU/mL), conjunctival wash/swab ($4.4 \pm 0.9 \log_{10}$ PFU/mL), and rectal swab ($3.6 \pm 0.3 \log_{10}$ PFU/mL) samples (Figure 2, panel A). The magnitude and frequency of viral titers in these specimens was reduced, but still present, in animals inoculated with a low dose of virus (Figure 2, panel B). Infectious virus was detected in either nasal or rectal wash samples in all contact animals on at least

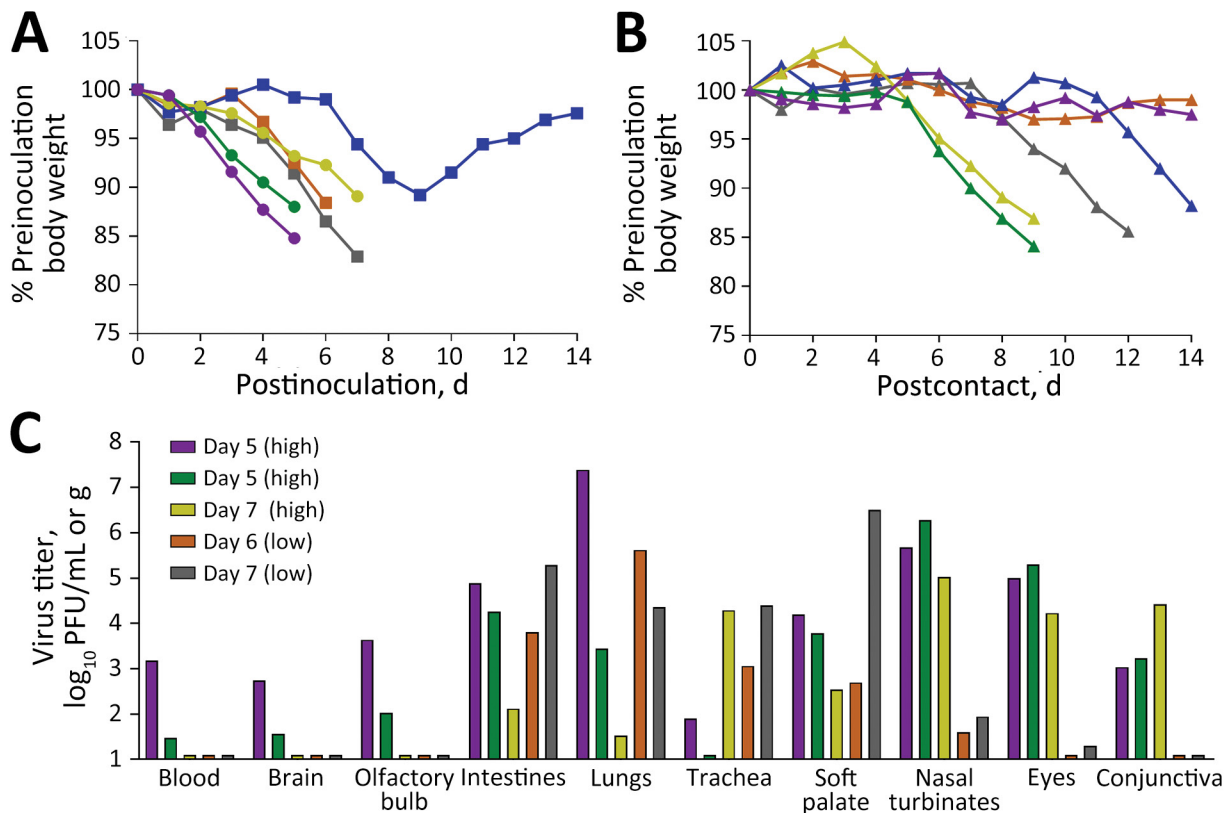


Figure 1. Disease severity and systemic spread of Chile/25945 influenza virus after ocular inoculation of ferrets. Ferrets were inoculated by the ocular route as previously described (7) with a high (10^6 PFU, circles) or low (10^3 PFU, squares) dose of Chile/25945 virus (100 μ L volume), and each was cohoused with a serologically naive ferret 24 hours after inoculation (triangles). A, B) Inoculated (A) and contact (B) animals were weighed daily and humanely euthanized after reaching previously described endpoints (3). Ferret inoculated:contact pairs are indicated with shared colors. C) Systemic tissues were collected from inoculated animals that reached humane endpoints and titered for the presence of infectious virus as previously described (7). Bars represent individual ferrets with the postinoculation day on which humane endpoints were reached and tissues were collected specified per inoculation dose (bar color is linked with ferret morbidity data shown in panel A). Limit of detection was 10 PFU.

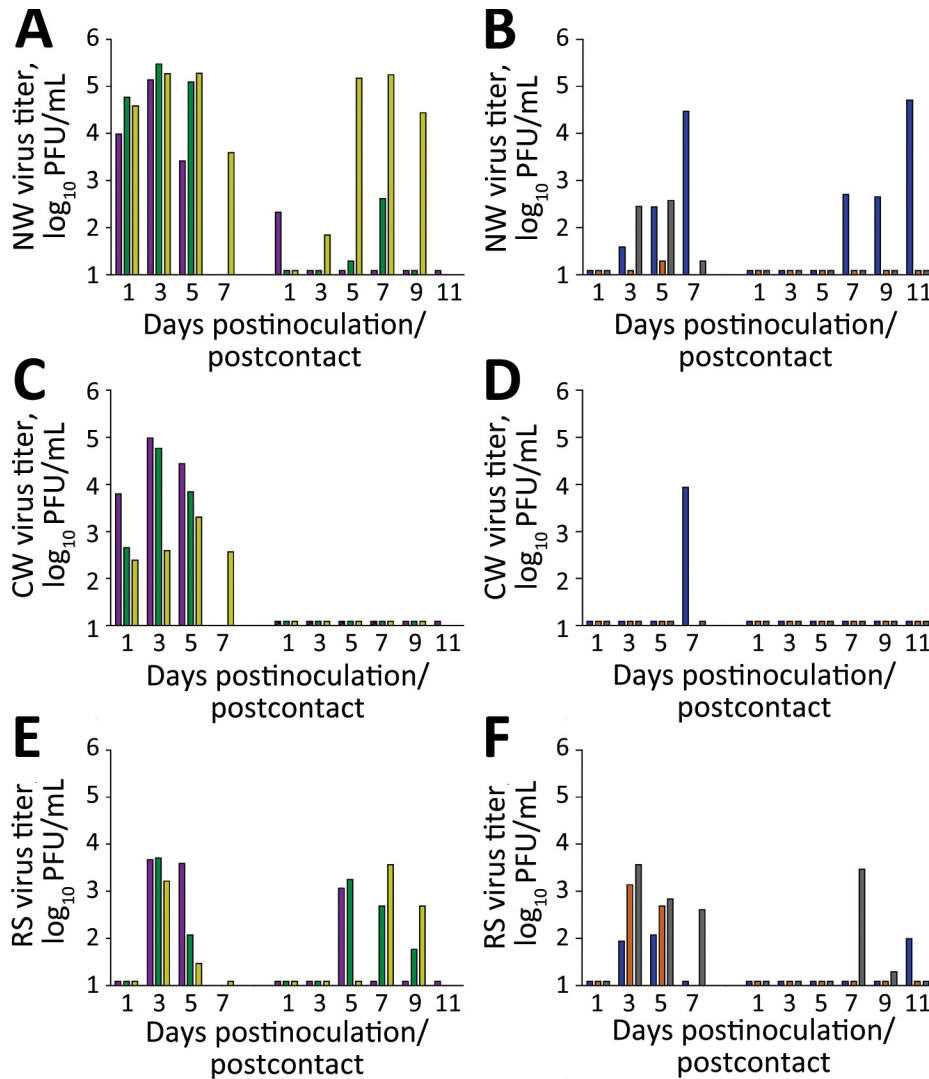


Figure 2. Transmission of Chile/25945 virus after ocular inoculation of ferrets. Ferrets were inoculated by the ocular route as previously described (7) with a high (10^6 PFU) or low (10^3 PFU) dose of Chile/25945 virus (100 μ L), and each was cohoused with a serologically naive ferret 24 hours after inoculation. Specimens were collected from all ferrets as previously described (7) on alternate days after contact. A) NW specimen after ferret inoculation with 10^6 PFU challenge dose; B) NW specimen after inoculation with 10^3 PFU challenge dose; C) CW specimen after ferret inoculation with 10^6 PFU challenge dose; D) CW specimen after ferret inoculation with 10^3 PFU challenge dose; E) RS specimen after ferret inoculation with 10^6 PFU challenge dose; F) RS specimen after ferret inoculation with 10^3 PFU challenge dose. On each graph, left-hand bars indicate inoculated ferrets and right-hand bars indicate contact ferrets. Absence of a bar indicates an animal was humanely euthanized and no specimen was collected. Bar colors are linked with ferret morbidity data shown in Figure 1, panels A, B. Limit of detection was 10 PFU. CW, conjunctival wash/swab sample; NW, nasal wash sample; RS, rectal swab sample.

1 day; infectious virus was not detected in conjunctival wash samples from any contact animal, possibly resulting from less overall virus shedding than in inoculated animals. Humane euthanasia because of severe disease was warranted for 4/6 contact animals (Figure 1, panel B); the other 2 survived, 1 of which exhibited a low level of seroconversion (hemagglutination inhibition titer 20).

Our finding that a clade 2.3.4.4b H5N1 virus isolated from a human can exhibit a virulent and transmissible phenotype after nontraditional inoculation, even with a low dose of inoculum, underscores the public health threat posed by those IAVs. The ocular surfaces may be exposed to infectious virus from the environment by several means (e.g., airborne particles, physical transfer from contact with fomites, and splashing liquids). Furthermore, circulation of tear fluid between ocular and nasopharyngeal tis-

sues via the lacrimal duct offers an opportunity for infectious virus to spread from the respiratory tract to the ocular system (8), in agreement with successful H5N1 viral isolation from both conjunctival and nasopharyngeal swab specimens from a human with conjunctivitis (5). Conjunctivae may be exposed to virus by direct contact (e.g., virus-contaminated hands), indirect contact (e.g., virus-contaminated fomites), or after deposition of virus-laden droplets or aerosols onto the ocular surface (4), permitting opportunities for H5N1 virus to establish a productive infection in humans even in the absence of an ocular tropism. Considering the myriad ways humans may be exposed to IAVs, our study supports the need to consider nontraditional inoculation modalities in risk assessment activities and supports consideration of using eye protection in potentially contaminated environments (9).

About the Author

Dr. Belser is a microbiologist in the Influenza Division, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, GA. Her research interests include the pathogenicity, transmissibility, and tropism of influenza viruses, with an emphasis on ocular tropism and ocular exposure.

References

1. Xie R, Edwards KM, Wille M, Wei X, Wong SS, Zanin M, et al. The episodic resurgence of highly pathogenic avian influenza H5 virus. *Nature*. 2023;622:810–7. <https://doi.org/10.1038/s41586-023-06631-2>
2. Centers for Disease Control and Prevention. Technical report: highly pathogenic avian influenza A(H5N1) viruses [cited 2024 Jun 2]. https://www.cdc.gov/flu/avianflu/spotlights/2023-2024/h5n1-technical-report_april-2024.htm
3. Pulit-Penalosa JA, Brock N, Belser JA, Sun X, Pappas C, Kieran TJ, et al. Highly pathogenic avian influenza A(H5N1) virus of clade 2.3.4.4b isolated from a human case in Chile causes fatal disease and transmits between co-housed ferrets. *Emerg Microbes Infect*. 2024 Mar 17:2332667. <https://doi.org/10.1080/22221751.2024.2332667>
4. Belser JA, Lash RR, Garg S, Tumpey TM, Maines TR. The eyes have it: influenza virus infection beyond the respiratory tract. *Lancet Infect Dis*. 2018;18:e220–7. [https://doi.org/10.1016/S1473-3099\(18\)30102-6](https://doi.org/10.1016/S1473-3099(18)30102-6)
5. Uyeki TM, Milton S, Abdul Hamid C, Reinoso Webb C, Presley SM, Shetty V, et al. Highly pathogenic avian influenza A(H5N1) virus infection in a dairy farm worker. *N Engl J Med*. 2024;NEJMc2405371. <https://doi.org/10.1056/NEJMc2405371>
6. Kandeil A, Patton C, Jones JC, Jeevan T, Harrington WN, Trifkovic S, et al. Rapid evolution of A(H5N1) influenza viruses after intercontinental spread to North America. *Nat Commun*. 2023;14:3082. <https://doi.org/10.1038/s41467-023-38415-7>
7. 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>
8. Olofsson S, Kumlin U, Dimock K, Arnberg N. Avian influenza and sialic acid receptors: more than meets the eye? *Lancet Infect Dis*. 2005;5:184–8. [https://doi.org/10.1016/S1473-3099\(05\)70026-8](https://doi.org/10.1016/S1473-3099(05)70026-8)
9. Centers for Disease Control and Prevention. Prevention and antiviral treatment of bird flu viruses in people [cited 2024 Jun 2]. <https://www.cdc.gov/flu/avianflu/prevention.htm>

Address for correspondence: Jessica A. Belser, Centers for Disease Control and Prevention, Mailstop H17-5, 1600 Clifton Rd NE, Atlanta, GA 30329-4018, USA; email: jbelser@cdc.gov

Genomic Epidemiology of Large Blastomycosis Outbreak, Ontario, Canada, 2021

Lisa R. McTaggart, Nobish Varghese, Karthikeyan Sivaraman, Samir N. Patel, Julianne V. Kus

Author affiliations: Ontario Agency of Health Protection and Promotion (Public Health Ontario), Toronto, Ontario, Canada (L.R. McTaggart, N. Varghese, K. Sivaraman, S.N. Patel, J.V. Kus); University of Toronto, Toronto (S.N. Patel, J.V. Kus)

DOI: <http://doi.org/10.3201/eid3007.231594>

Using phylogenomic analysis, we provide genomic epidemiology analysis of a large blastomycosis outbreak in Ontario, Canada, caused by *Blastomyces gilchristii*. The outbreak occurred in a locale where blastomycosis is rarely diagnosed, signaling a possible shift in geographically associated incidence patterns. Results elucidated fungal population genetic structure, enhancing understanding of the outbreak.

North American blastomycosis is an infection most commonly caused by environmental dimorphic fungi *Blastomyces dermatitidis* and *B. gilchristii*. Infections range from asymptomatic to severe, typically presenting as respiratory illness, with possible systemic dissemination (1,2). The geographic range of *B. dermatitidis* and *B. gilchristii* fungi spans the eastern half of North America, including Ontario, Canada (2,3). Although overall incidence rates are low, isolated cases of blastomycosis are diagnosed regularly among populations in endemic areas (2,3), and clusters and outbreaks occur due to common environmental exposures (1,4–7).

We describe a large genomic epidemiology investigation of a blastomycosis outbreak in Constance Lake First Nation, a small community (population <2,000) in northeastern Ontario, Canada, in a locale where blastomycosis has rarely been encountered (2). We studied samples from 181 patients that were received by the Public Health Ontario Laboratory during November 2021–May 2022. By August 2022, we identified *B. gilchristii* fungus, by using multilocus sequence typing (8), in cultures from 40 persons linked to the outbreak (37 community residents and 3 persons [deemed travel A, B, and C] who visited the community during the possible exposure window). We observed that most positive cultures (35/40) were derived from specimens collected during a 7-week period—mid-November 2021 through December