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Influenza Virus Infectivity and Virulence following Ocular-Only Aerosol Inoculation of Ferrets

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ABSTRACT

Respiratory pathogens have traditionally been studied by examining the exposure and infection of respiratory tract tissues. However, these studies typically overlook the role of ocular surfaces, which represent both a potential site of virus replication and a portal of entry for the establishment of a respiratory infection. To model transocular virus entry in a mammalian species, we established a novel inoculation method that delivers an aerosol inoculum exclusively to the ferret ocular surface. Using influenza virus as a representative respiratory pathogen, we found that both human and avian viruses mounted productive respiratory infections in ferrets following ocular-only aerosol inoculation, and we demonstrated that H5N1 virus can result in a fatal infection at doses below 10 PFU or with exposure times as short as 2 min. Ferrets inoculated by the ocular aerosol route with an avian (H7N7, H7N9) or human (H1N1, H3N2v) virus were capable of transmitting the virus to naïve animals in direct-contact or respiratory-droplet models, respectively. Our results reveal that ocular-only exposure to virus-containing aerosols constitutes a valid exposure route for a potentially fatal respiratory infection, even for viruses that do not demonstrate an ocular tropism, underscoring the public health implications of ocular exposure in clinical or occupational settings.

IMPORTANCE

In the absence of eye protection, the human ocular surface remains vulnerable to infection with aerosolized respiratory viruses. In this study, we present a way to inoculate laboratory mammals that excludes respiratory exposure, infecting ferrets only by ocular exposure to influenza virus-containing aerosols. This study demonstrates that the use of respiratory protection alone does not fully protect against influenza virus exposure, infection, and severe disease.

Influenza virus is considered primarily a respiratory pathogen: human infection results from respiratory exposure to the virus, and respiratory symptoms are the primary manifestation of disease. However, influenza virus receptors are not limited to respiratory tract tissues. Corneal and conjunctival epithelial cells also possess glycoconjugates bearing terminal sialic acids, as does the lining of the nasolacrimal duct, which provides an anatomical bridge between the respiratory and ocular systems (1, 2). Numerous respiratory pathogens, including influenza virus, adenovirus, and respiratory syncytial virus (RSV), have been shown to replicate specifically within ocular tissue and to use the eye as a gateway for the establishment of a productive respiratory infection, even if they do not typically demonstrate an ocular tropism in humans (1).

Mammalian models serve a critical public health role for the study of virus pathogenicity and transmissibility. For these purposes, small mammals are typically inoculated with respiratory viruses by intranasal (i.n.) instillation of a liquid virus suspension, although aerosol inhalation (AR) inoculation models, which deliver aerosol particles in the size range generated during coughing and sneezing, so as to better reflect natural exposure, have been developed recently (3). However, laboratory models of ocular inoculation have been limited to liquid instillation or intrastromal ocular delivery (1), methods that do not represent most human exposures. While human experimental studies have reported ocular spread of aerosolized influenza virus to the respiratory tract (4), not all viruses examined have demonstrated this capacity (5). Therefore, for all respiratory pathogens, rigorous assessment of the ability of ocular exposure to cause human infection is needed.

CDC guidelines recommend ocular protection in conjunction with respiratory protection during laboratory or occupational exposure to respiratory viruses (6), but these recommendations are not stringently observed, and numerous cases of ocular complications have been reported for individuals wearing respiratory protection in the absence of eye protection (1, 7, 8). Despite this, the relative risk of acquiring a respiratory infection via ocular exposure has not been examined. Using influenza virus as a model respiratory pathogen, we established a novel inoculation method that delivers a virus aerosol inoculum exclusively to the ferret ocular surface in order to examine the infectivity, virulence, and transmissibility of several human and avian influenza viruses. We found that an aerosolized highly pathogenic avian influenza (HPAI) virus delivered to the mammalian ocular surface was capable of causing severe and lethal disease in ferrets and could mount a productive respiratory infection capable of transmission to naïve contact animals. These results highlight the ocular epithelia as a poorly studied site of respiratory virus exposure and one that must be protected during potential exposure to aerosolized pathogens.

MATERIALS AND METHODS

Viruses. The influenza A viruses used in this study are shown in Tables 1 and 2. Virus stocks were propagated in the allantoic fluid cavities of 10-

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TABLE 1 Infectivity and pathogenesis of A/Thailand/16/04 virus in ferrets inoculated by the ocular aerosol route

Exposure time (min)	Virus dilution[a]	Ocular dose (PFU)[b]	Clinical signs and symptoms through day 14 p.i.				Virus detection through day 9 p.i.			
			Wt loss[c]	Fever[d]	Neuro[e]	Lethality[f]	NW[g]	CW[h]	RS[i]	Seroconv[j]
30	1:10	420–920	14.3 (3/3)	3.1 (3/3)	3/3	3/3 (7, 7, 8)	3.0 ± 1.1 (3–7)	1/3 (5–7)	2/3	NA
30	1:100	90–140	10.9 (2/2)	3.4 (2/2)	1/2	2/2 (5, 7)	3.4 ± 1.0 (3–7)	2/2 (3–7)	2/2	NA
30	1:1,000	9.6[k]	11.4 (1/1)	3.5 (1/1)	0/1	1/1 (7)	3.0 (5)	1/1 (5)	0/1	NA
10	1:10	238	11.5 (1/1)	2.8 (1/1)	0/1	1/1 (5)	2.4 (3)	0/1	0/1	NA
5	1:10	52–124[l]	11.0 (1/2)	2.1 (1/2)	1/2	1/2 (5)	4.7 (5)	1/2 (3–5)	1/2	1/2
2	1:10	17.6–50	13.0 (2/2)	2.7 (2/2)	1/2	2/2 (7, 8)	4.0 ± 0.5 (5–7)	1/2 (5)	1/2	NA

[a] Starting dilution of Thai/16 virus in nebulizer. The stock titer was 4×10^8 PFU/ml.
[b] Calculated as the virus concentration in the aerosol passed over the ocular surface multiplied by the exposure time.
[c] Mean maximum weight loss, expressed as a percentage, among ferrets with positive virus detection (number of ferrets with positive virus detection/total number of ferrets tested).
[d] Mean maximum rise in body temperature, in degrees centigrade, among ferrets with positive virus detection (number of ferrets with positive virus detection/total number of ferrets tested). The baseline temperature range was 37.3 to 39.1°C.
[e] Number of ferrets exhibiting neurological clinical signs (including hind limb paresis and torticollis)/total number of ferrets tested.
[f] Number of ferrets that succumbed to infection or were euthanized/total number of ferrets tested (with the days of death given in parentheses).
[g] Peak mean nasal wash (NW) titer, expressed as $\log_{10}$ PFU/ml ± standard deviation (with the day range p.i. of peak viral titer given in parentheses).
[h] Number of ferrets with positive virus detection in conjunctival wash (CW) specimens/total number of ferrets tested (with the day range p.i. of virus detection given in parentheses).
[i] Number of ferrets with positive virus detection in rectal swab (RS) specimens/total number of ferrets tested.
[j] Number of surviving ferrets that seroconverted to homologous virus by HI assay by the end of the experiment/total number of ferrets tested. NA, not applicable, because no ferrets survived to the end of the challenge period.
[k] One additional ferret received an ocular dose of 5.2 PFU but did not shed virus in NW specimens and did not seroconvert; it is excluded from this analysis.
[l] Two additional ferrets received ocular doses of 68 and 118 PFU but did not shed virus in NW specimens and did not seroconvert; they are excluded from this analysis.

day-old embryonated hens' eggs as described previously (9). Virus stock titers were determined by standard plaque assays using Madin-Darby canine kidney (MDCK) cells as described previously (10) and were expressed in PFU. All experiments were conducted under biosafety level 3 containment, including enhancements as required by the U.S. Department of Agriculture and the Federal Select Agent Program (11).

OA inoculation of ferrets. Male Fitch ferrets (Triple F Farms), 6 to 9 months old and serologically negative by hemagglutination inhibition (HI) assay to currently circulating influenza viruses, were used in this study. Ferrets were housed in a Duo-Flow BioClean mobile environmental enclosure (Lab Products, Seaford, DE) for the duration of each experiment. For ocular-only aerosol (OA) inoculations, ferrets were sedated as described previously (9) and were fitted with goggles with a neoprene gasket lining each eyepiece (Speedo), with holes drilled on the sides of each orbit to allow ¼-inch plastic tubing to feed into and out of each eyepiece. The goggles were secured to the ferret using self-adhering wrap, and the ferret was immediately placed in a secondary exposure chamber (Fig. 1). Each exposure session was conducted at 21°C and at 50% relative humidity for 2 to 30 min (the minimum and maximum exposure times possible due to airflow balancing of the aerosolization equipment and the duration of ferret anesthesia), followed by a 5-min purge to allow evacuation of the aerosolized virus from the chamber; ferrets were then removed and were returned to the holding cage. The ocular dose presented to each ferret was calculated by multiplying the concentration of virus in the aerosol (a function of the sampler virus concentration and volume in relation to the flow rate of the sampler and the time of exposure) by the exposure time. Air from the secondary chamber was continually collected throughout the duration of virus exposure with a biosampler identical to the primary sampler, with identical efficiency. Estimation of any potential inadvertent respiratory exposure was calculated identically to the ocular presented dose by using data from the secondary sampler; any ferret for which this respiratory presented dose was found to be ≥1 PFU was removed from analysis. Ferrets were monitored daily postinoculation (p.i.) for morbidity and clinical signs of infection (including body temperature, weight, and activity level) for at least 14 days as described previously (9). Any ferret that lost >25% of its preinoculation body weight or exhibited neurological dysfunction was euthanized and was subjected to postmortem examination. Virus shedding was measured on alternate days p.i. in nasal wash

TABLE 2 Pathogenesis of influenza viruses in ferrets inoculated by the ocular aerosol route

Virus	Name in this study	Subtype	Ocular dose (PFU)[a]	Clinical signs through day 12 p.i.		Virus detection through day 9 p.i.		
				Wt loss[b]	Fever[c]	NW[d]	CW[e]	RS[f]
A/Netherlands/219/03 (HPAI)	NL/219	H7N7	154–340	9.5 (8–12)	2.1	4.3 ± 0.4 (3–5)	0/3	2/3
A/Netherlands/230/03 (HPAI)	NL/230	H7N7	2.8–106[g]	9.1 (11–12)	2.1	4.2 ± 1.0 (3–5)	0/3	1/3
A/Mexico/InDRE7218/12 (HPAI)	Mex/7218	H7N3	14.6–198	11.1 (10–12)	2.7	5.2 ± 0.8 (5–7)	0/3	2/3
A/Shanghai/1/13 (LPAI)	Shanghai/1	H7N9	58–380	6.4 (2–12)	1.5	5.7 ± 0.5 (3)	2/3 (3–5)	0/3
A/Brisbane/59/07	Brisbane/59	H1N1	1.8–9.2	9.3 (6–9)	2.1	5.6 ± 0.1 (3–7)	3/3 (3–7)	0/3
A/Indiana/8/11	Indiana/8	H3N2v	156–500	8.9 (7–12)	1.4	5.6 ± 0.1 (3)	1/3 (3)	2/3

[a] Calculated as the virus concentration in the aerosol passed over the ocular surface multiplied by the exposure time (30 min).
[b] Mean maximum weight loss, expressed as a percentage, among ferrets with positive virus detection (with the day range p.i. of peak weight loss given in parentheses).
[c] Mean maximum rise in body temperature, in degrees centigrade, among ferrets with positive virus detection. The baseline temperature range was 37.5 to 38.8°C.
[d] Peak mean nasal wash (NW) titer, expressed as $\log_{10}$ PFU/ml ± standard deviation (with the day range p.i. of peak viral titer given in parentheses).
[e] Number of ferrets with positive virus detection in conjunctival wash (CW) specimens/total number of ferrets among all ferrets tested (with the day range p.i. of virus detection given in parentheses).
[f] Number of ferrets with positive virus detection in rectal swab (RS) specimens/total number of ferrets.
[g] One ferret received an ocular dose of 16.2 PFU but did not shed virus in NW specimens and did not seroconvert; it is excluded from this analysis.

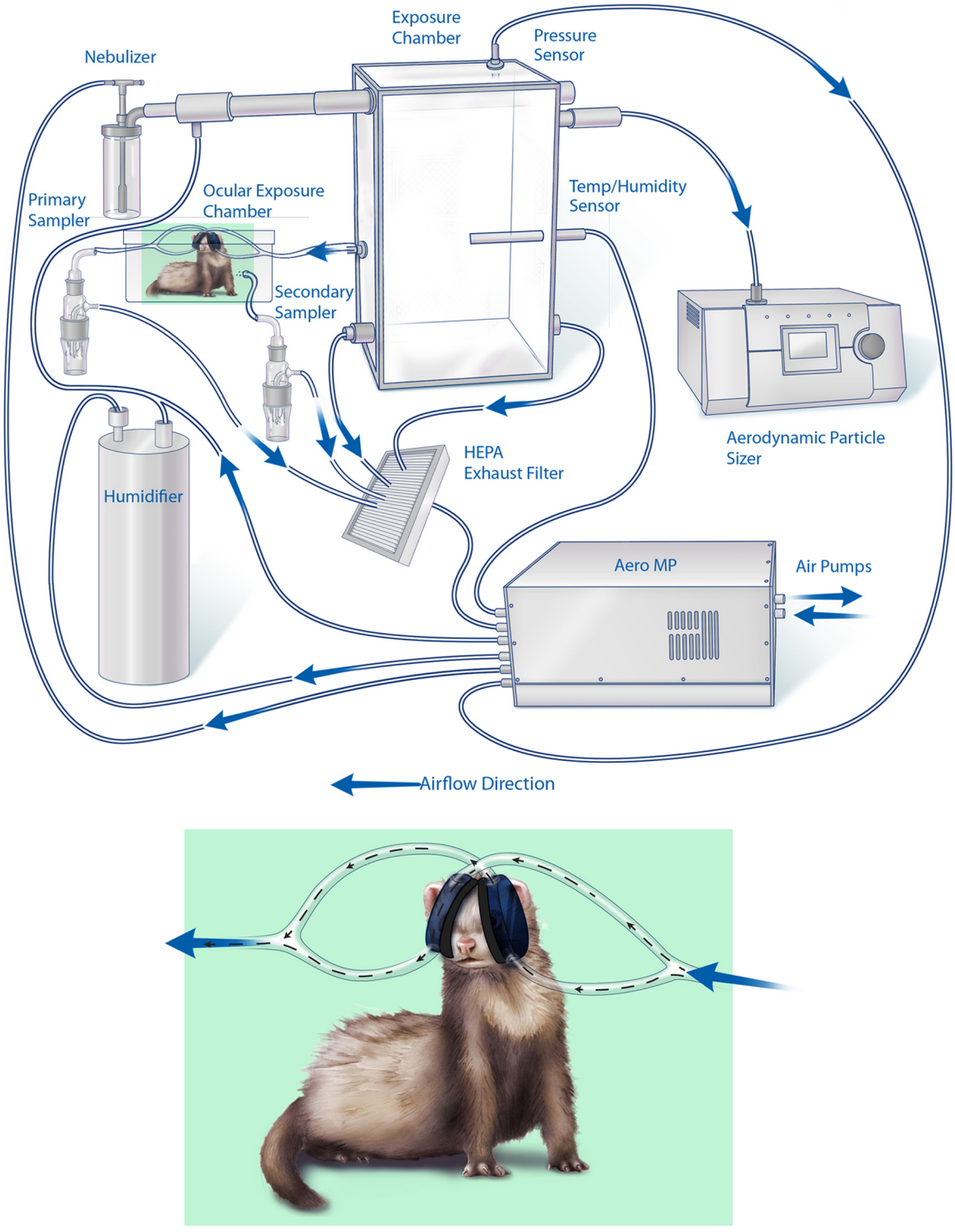

FIG 1 Graphical representation of ocular aerosol exposure system. An influenza virus aerosol system used for inhalation exposure of ferrets was modified to provide ocular-only aerosol exposure to ferrets. (Inset) Close-up of the aerosol delivery goggles used in ferret experiments.

(NW), conjunctival wash (CW), and rectal swab (RS) samples (12). All samples were titrated in MDCK cells by standard plaque assay as described previously (10). Animal research was conducted in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International, under the guidance of the Institutional Animal Care and Use Committee of the Centers for Disease Control and Prevention.

Transmission experiments. Virus transmissibility following OA challenge was assessed by placing a naïve ferret in the same cage as an inocu-

lated ferret (to assess transmission in the presence of direct contact [DC]) or in an adjacent cage with modified side walls to allow air exchange in the absence of direct or indirect contact between animals (to assess transmission by respiratory droplets [RD]) as described previously (13). Serum was collected on days 17 to 21 p.i. or postcontact (p.c.) for the measurement of seroconversion by HI assays as described previously (13).

RESULTS

OA inoculation. To assess transocular infection with influenza virus in a small mammalian model, we modified a whole-body aerosol exposure system previously developed to deliver an inhaled aerosol inoculum to ferrets (14). To create ocular-only aerosol (OA) exposure, we added a secondary exposure chamber to hold a sedated ferret fitted with aerosol delivery goggles that form a protective seal around each eye and the surrounding conjunctiva (Fig. 1). Aerosolized influenza virus was passed through the goggles for varying times and at varying concentrations. To confirm that ferrets received exclusively ocular exposure, air was sampled in the secondary exposure chamber throughout the duration of inoculation, and any ferret that received a respiratory dose of ≥1 PFU was excluded from this study.

To assess influenza virus infectivity and virulence following OA challenge, we inoculated ferrets with A/Thailand/16/2004 (Thai/16) virus, an HPAI H5N1 virus that is 100% lethal at low doses (<10 PFU) in ferrets inoculated i.n. or by AR (14). Postinoculation (p.i.), ferrets were observed daily for clinical signs of illness, and nasal wash (NW) specimens were collected on alternate days for virus titration. With an exposure time held constant at 30 min, serial dilutions of Thai/16 virus were administered by the OA route to two to three ferrets per group. Ferrets inoculated by the OA route succumbed to infection by day 8 after exposure to estimated presented ocular doses as low as 9.6 PFU (Table 1). Varying the duration of exposure to aerosolized virus while holding the concentration of virus constant revealed that a 2-min OA exposure (≤50 PFU) was sufficient to infect and cause lethality. Peak viral titers in NW samples were generally comparable between ferrets receiving low (<100 PFU) or high (>100 PFU) doses of the Thai/16 inoculum by the OA route (Fig. 2A and B). However, peak viral loads in NW samples were $<10^5$ PFU in all ferrets, and 40% of ferrets had peak viral loads of $\leq 10^3$ PFU prior to death (Fig. 2A and B). Virus was detected in samples collected from ocular and intestinal sites at days 3 to 7 p.i. among ferrets receiving both high and low doses of virus (Table 1).

Independently of the presented dose or the duration of virus exposure, ferrets that became infected with Thai/16 virus exhibited signs of severe disease, including weight loss, fever, and neurological symptoms necessitating euthanasia (Table 1). The virus was detected throughout the respiratory tract among ferrets euthanized on days 5 to 8 p.i., with a reduced frequency in lower-respiratory-tract than in upper-respiratory-tract tissues (Fig. 2C). Systemic spread of the virus to extrapulmonary tissues, including the brain, eye, and conjunctiva, was also uniformly detected. These studies demonstrate that ocular-only exposure to aerosolized Thai/16 virus at doses below 10 PFU or for an exposure time as short as 2 min is sufficient to cause severe disease and death in ferrets.

Infectivities of human and avian influenza viruses following OA inoculation. While H7 subtype influenza viruses are frequently associated with conjunctivitis, humans are exposed to aerosols containing a diverse range of influenza viruses, the majority of which do not possess an ocular tropism. As such, the infectivity and virulence of several human and avian influenza viruses were assessed following 30-min OA inoculations at the doses indicated in Table 2. All influenza viruses tested were capable of mounting a productive respiratory infection following OA exposure, including avian influenza viruses associated with ocular disease in humans (NL/230, Mex/7218), avian viruses associated with severe respiratory disease in humans (NL/219, Shanghai/1),

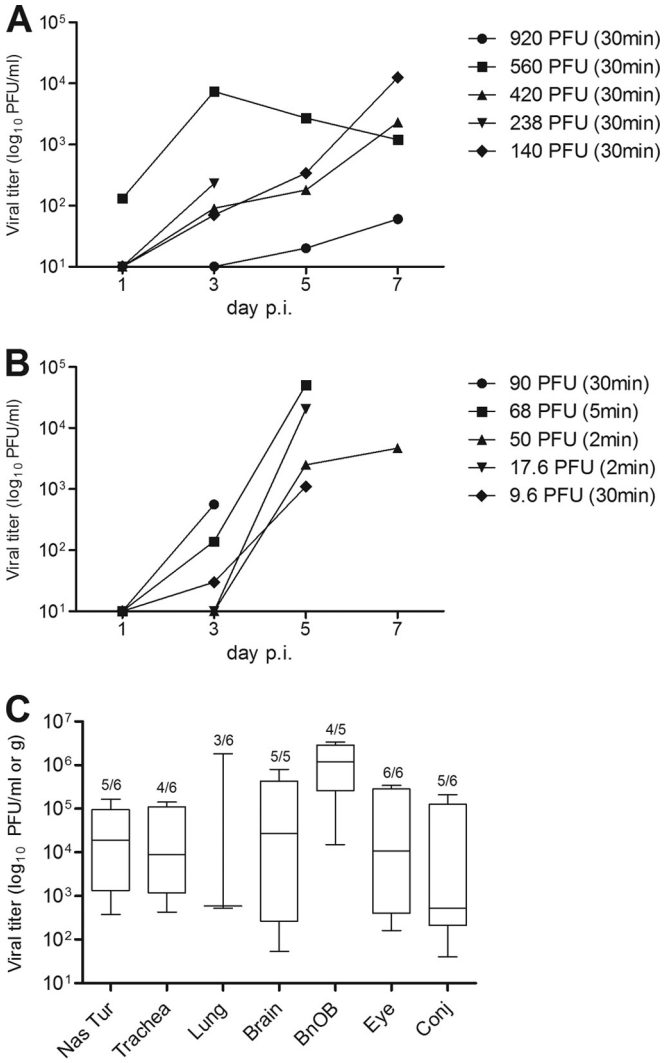

FIG 2 A/Thailand/16/04 viral titers in nasal wash specimens and tissues following OA inoculation. (A and B) Ferrets were inoculated by the OA route with a high (>100-PFU) (A) or low (<100-PFU) (B) dose of virus, and virus titers in nasal wash specimens were determined on the indicated days p.i. Each line represents an individual ferret receiving the indicated dose. The duration of exposure is given in parentheses in the key. (C) Box-and-whisker plots of titers in tissues collected from ferrets euthanized due to neurological symptoms on days 5 to 8 after inoculation with the Thai/16 virus. The mean numbers of ferrets with positive virus detection in each tissue are shown; the number of ferrets with positive virus detection out of the total number of ferrets sampled is given above each box-and-whisker plot. Nas Tur, nasal turbinates; BnOB, olfactory bulb; Conj, conjunctiva. Titers are expressed in PFU/g in all tissues except for Nas Tur, Eye, and Conj, in which titers are expressed in PFU/ml. Eye and conjunctiva samples include both left and right tissues. "Brain" includes both anterior and posterior segments. The limit of detection for all titrations was 10 PFU.

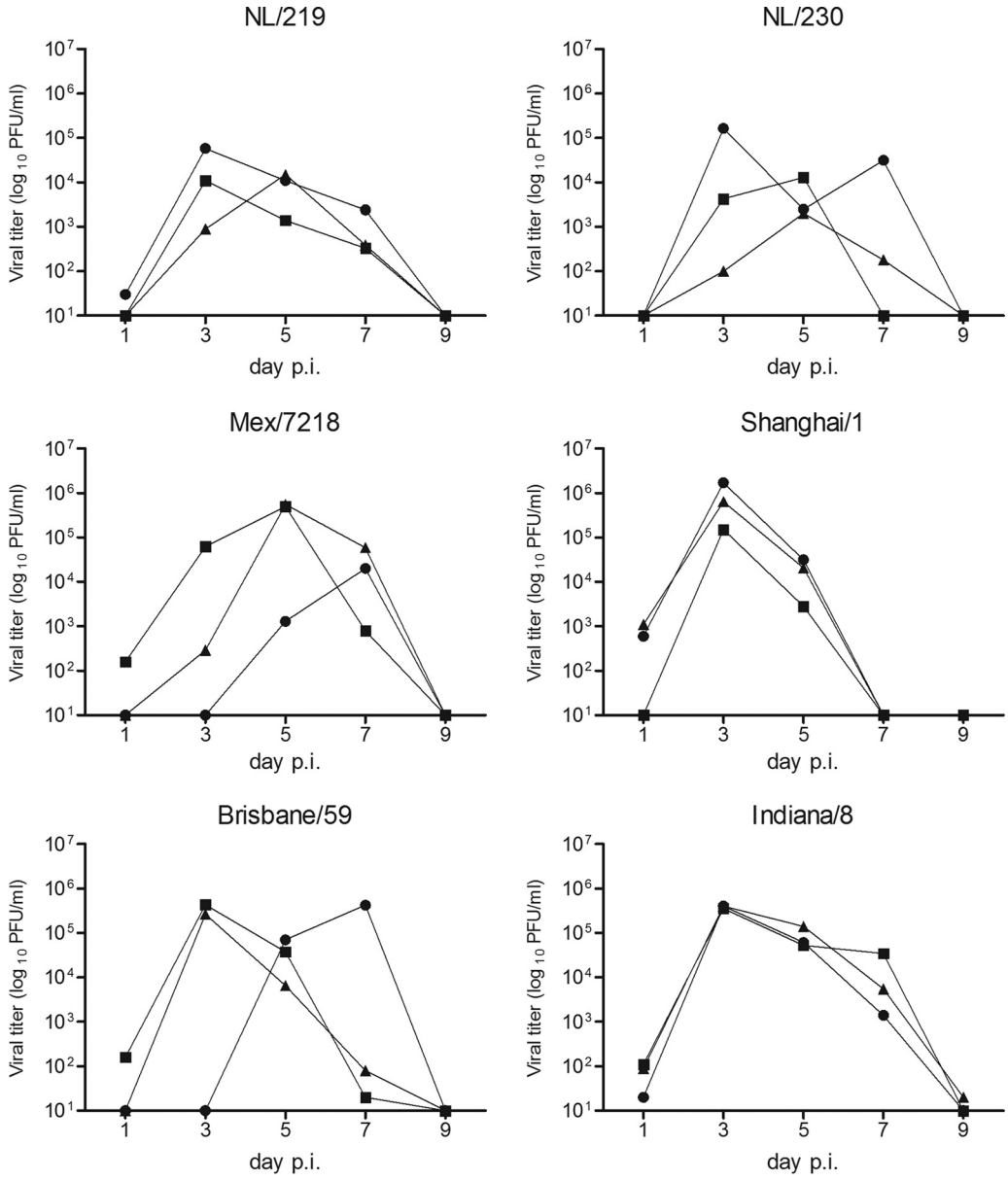

FIG 3 Titers of influenza viruses recovered from ferret nasal wash specimens following OA inoculation. Ferrets were inoculated by the OA route with the indicated viruses at the doses reported in Table 2. Viral titers were measured in nasal wash specimens collected on the indicated days p.i. Lines represent individual ferrets. The limit of detection was 10 PFU.

and former seasonal and H3N2 variant strains of human viruses (Brisbane/59, Indiana/8) (Table 2) (1, 15). Infectivity following OA exposure was high: all viruses infected ferrets at doses of <200 PFU, and a seasonal H1N1 virus was capable of infecting ferrets at doses of <10 PFU. Weight loss and fever were detected among all ferrets with positive virus isolation in NW samples (Table 2). The durations of virus shedding from NW samples in OA-inoculated ferrets were generally comparable for all strains; virus was typically detected in NW samples by day 3 p.i. before clearing by day 9 p.i. (Fig. 3), with sporadic detection of virus in ocular and intestinal sites (Table 2). No association between the dose of virus presented and the severity of infection was observed (Table 2; also data not shown), indicating that ferrets that became productively infected following OA exposure exhibited comparable disease progress and resolution.

Influenza virus transmissibility following OA inoculation. To determine if OA exposure to influenza virus resulted in a transmissible respiratory infection, we assessed the abilities of selected avian and human influenza viruses, known to be transmitted following traditional i.n. inoculation, to spread to naïve contact animals following OA inoculation. Transmission was assessed by placing ferrets in direct contact (DC), or separated in adjacent cages with perforated side walls to allow air exchange only (respiratory droplet [RD] transmission) (13). The HPAI H7N7 virus NL/230 and the low-pathogenicity avian influenza (LPAI) H7N9 virus Shanghai/1, associated with human conjunctivitis and severe respiratory disease, respectively, are capable of transmission between ferrets in direct contact when inoculated i.n. (16, 17). Following OA inoculation, both H7 viruses were transmitted to naïve ferrets in direct contact (Fig. 4A), spreading to 2/3 and 3/3 ferrets,

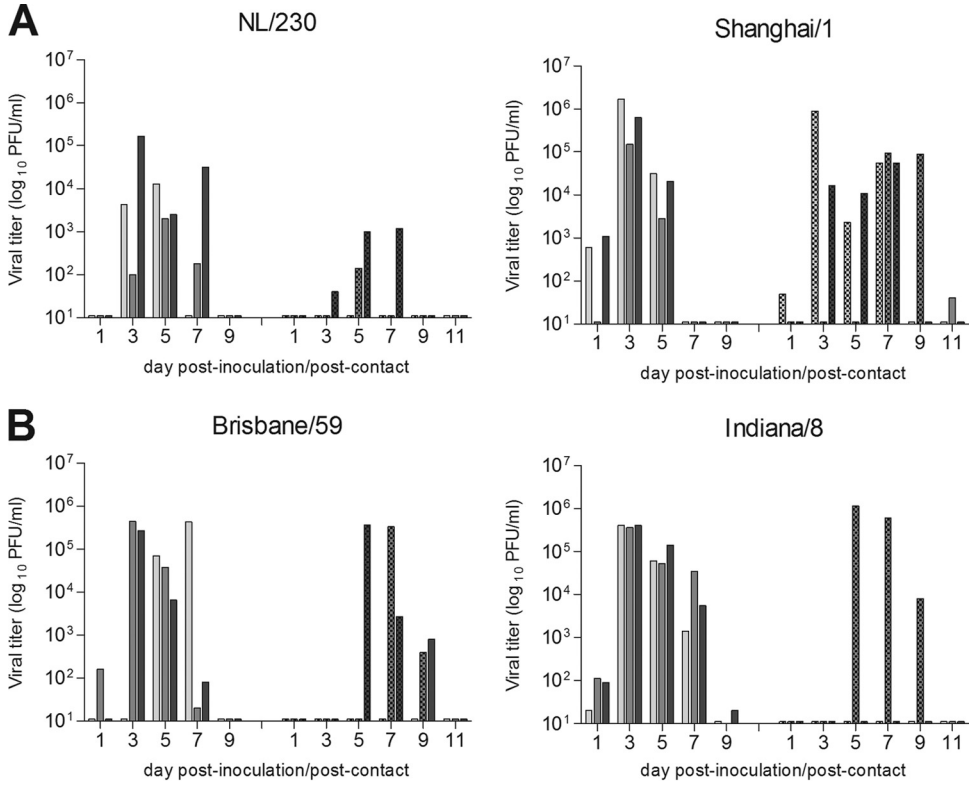

FIG 4 Transmissibilities of influenza viruses in ferrets following OA inoculation. Three ferrets were inoculated by the OA route with the indicated viruses at the doses reported in Table 2, and nasal wash specimens were collected from each ferret on the indicated days p.i. (solid bars). A naïve ferret was placed either in the same cage as each inoculated ferret (A) or in an adjacent cage with perforated side walls (B) at 24 h p.i., and nasal wash specimens were collected from each contact ferret on the indicated days p.c. (hatched bars) in order to assess virus transmission in the presence of direct contact or respiratory droplets, respectively. The limit of virus detection was 10 PFU.

respectively, by day 7 postcontact (p.c.); the third H7N7 contact ferret did not shed virus and remained seronegative at the end of the study (data not shown).

Next, we inoculated ferrets by the OA route with human influenza viruses and assessed transmission by the more stringent RD route. Both the H1N1 virus Brisbane/59 and the H3N2 variant virus Indiana/8 are transmitted efficiently in ferrets by RD when inoculated i.n. (15, 18). Both viruses replicated to titers of $>10^5$ PFU when ferrets were inoculated by the OA route and were capable of transmission by RD, albeit at a reduced frequency, with only 2/3 and 1/3 ferrets, respectively, shedding virus in NW specimens and seroconverting to the homologous virus (Fig. 4B). These findings nonetheless indicate that ferrets inoculated by the OA route with either avian or human influenza viruses maintain the capacity for respiratory spread to naïve animals.

DISCUSSION

Beyond the respiratory tract, human eye epithelia represent an additional mucosal surface possessing numerous cellular receptors employed by respiratory viruses contained in infectious aerosols and contaminated fomites (1, 2, 19). The nasolacrimal system further allows for respiratory pathogens to use the eye as a portal of entry to gain passage to extraocular tissues bearing permissive receptors. However, there is a paucity of data examining the ability of a respiratory virus to mount a productive infection following ocular exposure to virus-containing aerosols, and there is little understanding of how an ocular-only aerosol inoculation might modulate the virulence and transmissibility of the resulting infection relative to those with intranasal or inhalation challenges. In this study, we used influenza virus as a model pathogen to examine two scenarios of potential human ocular exposure: occupational or laboratory exposure to HPAI and LPAI viruses with pandemic potential and community-acquired exposure to seasonal influenza viruses, which circulate widely in human populations.

Small mammalian models (including mice, ferrets, rabbits, and cotton rats) have been established for the study of ocular inoculation of numerous respiratory viruses, including influenza virus, adenovirus, and RSV (1), but do not recapitulate potential ocular exposure to virus-containing aerosols expelled from infected individuals during breathing, coughing, or sneezing (20). Several mammalian models that utilize an aerosolized virus inoculum have been established to address this need but have been restricted to inhalation exposure (3). Ferrets are used for the study of numerous viral pathogens (21, 22) and were chosen to model ocular-only aerosol exposure because their physiology and distribution of cellular receptors in both respiratory and ocular tissues closely match those of humans (12, 21). Therefore, while influenza virus was used in this study, the OA inoculation method presented here could easily be adapted for parallel studies with other pathogens that pose a similar threat to human health.

Influenza virus infectivity following ocular aerosol exposure has not been investigated to date. The low ($<$10-PFU) doses of both avian and human influenza viruses presented to the ferret ocular surface, sufficient to mount a productive respiratory infec-

tion (Tables 1 and 2), indicate that the ocular infectious dose of influenza virus is comparable to that for i.n. or AR delivery (14). Furthermore, the comparable fatal outcomes for ferrets exposed to H5N1 virus by the OA route for 2 to 30 min suggest that prolonged ocular exposure to influenza virus-containing aerosols is not necessary for the mounting of a lethal infection (Table 1). It therefore appears that the replication-independent spread of virus from ocular to respiratory tissues reported previously with models of ocular inoculation (12, 23) is not time dependent or dose dependent and that the ocular surface is highly susceptible to low levels of aerosolized influenza virus. However, it does not appear that virus replication in ocular tissue is necessary for subsequent respiratory infection, because not all productively infected ferrets had detectable virus in ocular samples (Tables 1 and 2). As in other models of ocular inoculation with influenza viruses (12, 24), 100% infectivity of ferrets following OA inoculation was not observed, likely due to low presented doses, ferret-to-ferret physiological differences, or differences in the ocular surface area exposed during OA inoculation. The inclusion of an impinger sampler to measure the presence of aerosolized virus in the secondary chamber represents the most direct and comprehensive method possible to measure potential respiratory exposure during ocular-only inoculation; the absence of aerosolized virus outside the goggles during the inoculation and the lack of detection of virus in conjunctival wash specimens on day 1 p.i. indicate that it is unlikely that the aerosolized virus inoculum had persisted on the fur of the ferrets, leading to self-inoculation.

Limited reports of presumed human-to-human transmission among individuals infected by the ocular route suggest the potential for ocular exposure to lead to a transmissible infection in humans (25). In support of this, ferrets inoculated by the OA route were capable of both DC and RD transmission to naïve contacts (Fig. 4), albeit at a frequency lower than that for ferrets inoculated i.n. with high doses of virus (15–18). Lower challenge doses, delays in virus replication, and reduced levels of proinflammatory cytokine and chemokine transcripts in respiratory tract tissues from ferrets inoculated by the ocular route compared with the i.n. route suggest a potential mechanism for the reduced frequency of virus transmission observed in these studies (26). Accordingly, a decreased incidence of sneezing and nasal discharge was detected among ferrets inoculated by the ocular route (either with a liquid inoculum or by OA inoculation) (12), which could contribute to reduced transmissibility. Further study is needed to better understand the dissemination of virus from ocular tissues and the contribution this may make to virus transmission.

While OA inoculation of ferrets offers an effective way to model natural exposures of the ocular surface to virus that could result in a respiratory infection, it does not model macroscopic influenza virus-associated ocular disease. As in other studies, ferrets inoculated with influenza virus by the ocular route did not develop conjunctivitis or present with ocular complications (1). Infectious virus was recovered from ocular samples collected from selected OA-inoculated ferrets throughout the acute phase of infection (Tables 1 and 2; Fig. 2C), indicating that virus was present in this tissue. Therefore, OA inoculation of ferrets may represent an appropriate model for the study of the effects of vaccination and antiviral treatment in mitigating viral loads in ocular tissue following exposure to influenza viruses, especially strains that exhibit an ocular tropism in humans (27). Future study evaluating OA inoculation of respiratory viruses that do cause macroscopic ocular disease in mammalian models, such as RSV, is also warranted (1, 23).

Understanding the risk of ocular exposure to respiratory pathogens and the course of disease following infection by this route has numerous implications for both clinicians and public health officials. Using influenza virus as a model virus, we created a novel inoculation method that delivers a virus aerosol inoculum exclusively to the ferret ocular surface, and we have demonstrated that the eye represents a potential entry point for human and avian influenza viruses, which can then mount a productive and potentially fatal infection in ferrets in the absence of respiratory exposure. These findings allow for greater understanding of emerging influenza viruses and more-accurate assessment of the threat these viruses pose to human health, and they underscore the importance of eye protection during occupational exposure to aerosols containing influenza viruses of all subtypes.

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