Transmission of Influenza B Viruses in the Guinea Pig

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Epidemic influenza is typically caused by infection with viruses of the A and B types and can result in substantial morbidity and mortality during a given season. Here we demonstrate that influenza B viruses can replicate in the upper respiratory tract of the guinea pig and that viruses of the two main lineages can be transmitted with 100% efficiency between inoculated and naïve animals in both contact and noncontact models. Our results also indicate that, like in the case for influenza A virus, transmission of influenza B viruses is enhanced at colder temperatures, providing an explanation for the seasonality of influenza epidemics in temperate climates. We therefore present, for the first time, a small animal model with which to study the underlying mechanisms of influenza B virus transmission.

Influenza viruses are negative-strand RNA viruses of the family *Orthomyxoviridae*. The viruses can be divided into three types: influenza A, B, and C viruses. Although both influenza A and B viruses are known to cause severe disease in humans, differences exist in their host range, evolutionary rates, virion structure, and genomic organization (34, 53). Typically, either or both types of influenza viruses can cause epidemic disease in a given year.

Signs of uncomplicated influenza B virus infection include coughing, sore throat, nasal obstruction or discharge, fever, and chills (50, 53); influenza B complications such as secondary bacterial pneumonia can also occur. Reye syndrome following aspirin administration in the context of influenza B virus infection has also been well documented, with an increased association between Reye syndrome and influenza B virus infection compared to that for infection with influenza A virus (53).

While disease can occur in adults and the elderly (31, 49, 51), influenza B virus infection can be more severe in pediatric patients (2, 12, 17, 32). Nonrespiratory clinical outcomes, such as myositis and gastrointestinal complications, tend to occur more commonly in children (12, 50). In rare cases, influenza B virus infection has been associated with encephalitis (29). Bacterial coinfections with influenza B virus also pose a serious risk (6, 35, 39). Overall, substantial morbidity and mortality can be associated with influenza B virus infection in the pediatric population. From 2004 to 2010, 22 to 36% of pediatric influenza deaths were related to influenza B virus infection (3). During the 2010–2011 influenza season, 38% of laboratory-confirmed influenza-associated pediatric deaths reported in the United States were associated with influenza B viruses (6). Thus, influenza B virus can cause severe outcomes in infected patients, particularly in the case of children.

Influenza B viruses can be categorized into two distinct lineages: B/Victoria/2/1987-like (B/Victoria-like) and B/Yamagata/16/1988-like (B/Yamagata-like) viruses. For preparation of the influenza B virus component of the inactivated or cold-adapted trivalent vaccine, either a B/Victoria-like or B/Yamagata-like virus is selected (53). Viruses from the two influenza B virus lineages are antigenically distinct (16, 38), making vaccine preparation difficult, as viruses from both lineages can circulate in a given season (2, 3), sometimes even cocirculating within the same outbreak (17). Indeed, the influenza B virus strain selected to be incorporated into the trivalent vaccine was mismatched 50% of the time from 2001 to 2010, resulting in limited protection against the circulating strain during those years (2). An animal model system with which to study the efficacy of different influenza B virus

vaccines against various influenza B virus strains could therefore be helpful in the selection of the strain to be included in a new seasonal vaccine formulation.

There is a relative lack of animal models with which to study influenza B virus infection, in contrast to the case for influenza A virus infection. This is thought to be due to the limited host range of influenza B viruses, as these viruses predominantly infect humans (4, 30, 33). Nonstructural protein 1 (NS1) (9, 15, 43, 54) and the matrix protein (M1) (25) of influenza B viruses have been implicated in this phenotype.

Animal models, including guinea pig and ferret models, are used routinely to study the transmission of influenza A viruses (1, 5, 8, 19–22, 28, 41, 44, 46, 47). To our knowledge, influenza B virus transmission has not yet been demonstrated in any experimental animal model. Here we report that influenza B viruses not only replicate in the upper respiratory tract of guinea pigs but also are transmitted efficiently from infected to naïve animals under both contact and noncontact conditions. Furthermore, we demonstrate that the influenza B virus transmission phenotype shows a similar dependence on temperature to that documented for influenza A virus transmission. Thus, in the guinea pig model, transmission of both influenza A and B viruses is enhanced at cold temperatures, which may explain, at least in part, the seasonality of influenza epidemics in temperate climates. In summary, the guinea pig provides an opportunity to investigate other novel aspects of influenza B virus transmission in a small animal model.

MATERIALS AND METHODS

Cells and viruses. Madin-Darby canine kidney (MDCK) cells were maintained in minimum essential medium supplemented with 10% fetal bovine serum and antibiotics. Recent clinical influenza B virus isolates were obtained from the Centers for Disease Control and Prevention in Atlanta, GA. Virus stocks were prepared by inoculation of 10-day-old chicken eggs with 100 PFU of virus, followed by incubation at 33°C for 72 h. Stock titers were determined by plaque assay of 10-fold serial dilutions of allantoic fluid, as described below. In order to purify virus for enzyme-linked immunosorbent assays (ELISAs), virus stocks were grown in 10-day-old

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FIG 1 Day 2 postinoculation nasal wash titers of guinea pigs infected with influenza B viruses. Groups of four animals were inoculated intranasally with 10^4 PFU/ml of the following influenza B viruses: B/Victoria/2/1987, B/Malaysia/2506/2004, B/Florida/4/2006, and B/Brisbane/60/2008. Animals were anesthetized and nasal washes performed 2 days after inoculation. Virus titers were quantified by plaque assay. Horizontal bars indicate average nasal wash titers for the four inoculated animals.

chicken eggs and the allantoic fluid was harvested 72 h later. Virus was pelleted by centrifugation at 10,000 rpm for 30 min at 4°C, resuspended in $1 \times$ NTE buffer (0.5 mM NaCl, 10 mM Tris-HCl, pH 7.5, 5 mM EDTA), and then purified over a 30% sucrose cushion.

Animals. All animal experiments were performed in accordance with the guidelines of the Mount Sinai School of Medicine Institutional Animal Care and Use Committee. Five- to six-week-old female Hartley strain guinea pigs were obtained from Charles River Laboratories. Animals were ordered to be between 350 and 400 g. Animals were allowed access to food and water *ad libitum* and were kept on a 12-h light-dark cycle.

Infection and monitoring of guinea pigs. Prior to infection, guinea pigs were anesthetized with a 175- μ l mixture of ketamine (30 mg/kg of body weight) and xylazine (5 mg/kg) administered intramuscularly. An inoculum of 10^4 PFU was instilled intranasally by applying 150 μ l to each nostril as previously described (5, 19, 20). Body weight was measured once daily for initial experiments.

Collection of guinea pig nasal wash samples. Prior to nasal wash sample collection, guinea pigs were anesthetized as described above. Nasal washing was performed by instilling a total of 1 ml of phosphate-buffered saline (PBS) into the nostrils of a guinea pig and allowing it to drain into a sterile petri dish. Supernatants were stored at -80°C before analysis by plaque assay.

Collection and processing of guinea pig tissue samples. Guinea pigs were infected with either B/Victoria/2/1987 virus or B/Florida/4/2006 virus or were mock infected as described above. Animals were anesthetized with ketamine-xylazine as described above and then sacrificed with phenobarbital on day 2 or 4 postinfection ($n = 2$ /virus/time point), and nasal turbinates, the trachea, and lung tissues were harvested. Soft tissue samples were stored in formalin until they were embedded in paraffin. Samples collected from the nasal turbinates were first decalcified and then fixed with formalin and paraffin embedded. Sections were prepared and stained with hematoxylin and eosin (H&E) and then read by a veterinary pathologist.

Guinea pig transmission experiments. All transmission experiments were performed as previously described (5, 19, 20). Infected guinea pigs were isolated from naïve animals for the first day following intranasal inoculation. On day 1, four transmission pairs were set up in environmental chambers (Caron model 6030) that maintain temperature and humidity, with each pair consisting of one virus-infected guinea pig and one naïve guinea pig. For contact experiments, animal pairs were cocaged. For respiratory droplet experiments, animals were placed in separate cages that had the plastic from one side of the cage removed and replaced with a wire mesh that allowed air to flow between cages. Nasal wash samples were collected from exposed and infected animals at 2, 4, 6, and 8 days postinfection (dpi). An additional nasal wash was collected from exposed animals at 10 dpi. During transmission experiments, strict measures were followed in order to prevent cross-contamination as a result of animal handling. Exposed animals were handled prior to the handling of infected animals. Gloves and work surfaces were sanitized between the handling of

each animal pair. Animals were anesthetized again on day 30, at which point blood was collected by terminal cardiac puncture and animals were sacrificed immediately under anesthesia by CO_2 inhalation. Serum was separated from blood cells by centrifugation and stored at -20°C .

Quantification of viral titers. Virus stock and nasal wash titers were determined by plaque assay of 10-fold serial dilutions in PBS-bovine serum albumin (BSA)-penicillin-streptomycin on MDCK cells in the presence of 1 $\mu\text{g/ml}$ tosylsulfonyl phenylalanyl chloromethyl ketone (TPCK)-trypsin. Cells were incubated for 72 h at 33°C . Cells were then fixed with 4% formaldehyde, and plaques were visualized with a crystal violet counterstain. For quantification of B/Florida/4/2006 virus, plaques were visualized by immunostaining as described previously (23, 45). For all experiments, a transmission event was defined to occur when the nasal wash titer for an exposed guinea pig was above 10 PFU/ml.

ELISA. Sera from guinea pigs were separated from red blood cells by centrifugation and stored at -20°C . Wells of ELISA plates (Immulon 4 HBX) were coated with 50 μl of purified virus at a concentration of 2 $\mu\text{g/ml}$ in PBS following an overnight incubation at 4°C . Virus was then removed, and plates were blocked with 1% BSA in PBS for 1 h at room temperature. Following this incubation, plates were washed and dilutions of guinea pig sera were added to the plate. Plates were then washed again and incubated with 50 μl of alkaline phosphatase-linked anti-guinea pig secondary antibody (Abcam) for 1 h at room temperature. Fifty microliters of *p*-nitrophenyl phosphate substrate (Invitrogen) was then added after plates were washed a final time. After 30 min, the reaction was stopped with 50 μl of 0.75 N NaOH, and the plates were read at 405 nm in an ELISA plate reader (DTX 880 multimode detector; Beckman Coulter).

Statistical tests. To assess statistical differences in viral titers for inoculated guinea pigs, one-tailed unpaired Student's *t* test was used. Welch's correction was applied when variances were calculated to be statistically different. To assess statistical differences in the "last day of high titer," a one-tailed Mann-Whitney (nonparametric) test was used. Area under the curve (AUC) values were calculated for each individual guinea pig, and then the statistical difference between the means was assessed by Student's *t* test as described above. All statistical analyses were performed in Prism4 (GraphPad Software).

RESULTS

Influenza B viruses replicate in the upper respiratory tract of the guinea pig. In order to determine whether influenza B viruses were capable of replicating in guinea pigs, groups of four animals were inoculated with 10^4 PFU of recent influenza B virus isolates representing the B/Victoria-like (B/Malaysia/2506/2004 and B/Brisbane/60/2008 viruses) and B/Yamagata-like (B/Florida/4/2006 virus) lineages, as well as B/Victoria/2/1987 virus. At 2 dpi, nasal washes were collected from each animal and plaque assays

FIG 2 Histological changes following influenza B virus infection in the guinea pig respiratory tract. Animals were infected with B/Victoria/2/1987 or B/Florida/4/2006 virus or mock infected with PBS. On days 2 and 4, animals were sacrificed and tissue samples were collected ($n = 2$ /virus/time point). (A) H&E staining of the epithelium lining the nasal septum, of the trachea, and of lung sections of B/Victoria/2/1987 virus-infected guinea pigs was on the whole unremarkable and was similar to what was seen in mock-infected animals on day 2 postinfection. B/Florida/4/2006 virus-infected guinea pigs displayed cellular infiltration and moderate goblet cell hyperplasia in tracheal samples. In the lungs, moderate mononuclear and heterophilic infiltration was observed, with alveolar thickening. (B) B/Victoria/2/1987 virus-infected samples were similarly unremarkable on day 4, with the exception of some goblet cell hyperplasia in the trachea of one of the infected animals (pictured). B/Florida/4/2006 virus-infected guinea pigs showed more dramatic histopathological changes. Cellular infiltration and goblet cell hyperplasia were observed in the tracheal samples of both of the infected guinea pigs. In the lungs, multifocal mononuclear and heterophilic infiltration, as well as alveolar thickening, was seen. Mild alveolitis was seen in one animal at this time point.

were performed. Influenza B viruses replicated to high titers in the nasal passages of each guinea pig (Fig. 1), though it is noteworthy that overt clinical signs of infection were not observed in this 2-day monitoring period.

Influenza B virus infection results in histological changes in the upper and lower respiratory tracts of the guinea pig. Guinea pigs were infected with representative influenza B viruses from the B/Victoria (B/Victoria/2/1987 virus) or B/Yamagata (B/Florida/4/

FIG 3 Transmission of B/Victoria/2/1987 virus from inoculated guinea pigs to naïve cocaged animals at 5°C and 20°C. Four animals were inoculated intranasally with 10^4 PFU of B/Victoria/2/1987 virus. Each of these animals was placed in a cage with one naïve guinea pig on the following day. Nasal washes were performed on days 2, 4, 6, and 8 postinoculation. An additional nasal wash was performed on exposed animals on day 10. One hundred percent transmission occurred at both 20°C with 20% relative humidity (A) and 5°C with 20% relative humidity (B). Inoculated animals are represented by solid lines; exposed animals are represented by dotted lines. Guinea pig pairs are denoted by the following symbols: squares, pair 1; triangles, pair 2; octagons, pair 3; and diamonds, pair 4. (C) Seroconversion was assessed by ELISA, with serum from a guinea pig infected with an influenza A (H1N1) virus as a negative control.

2006 virus) lineage or mock infected and then were sacrificed on day 2 or 4 postinoculation. B/Victoria virus-infected animals showed little histological changes relative to mock-infected animals throughout the respiratory tract on both day 2 and day 4. Goblet cell hyperplasia and heterophilic infiltration were observed in the trachea of only one infected animal, on day 4 (Fig. 2). (The guinea pig equivalent of the human neutrophil is called a heterophil.)

Histopathological changes were more pronounced in animals infected with B/Florida/4/2006 virus. While H&E staining of nasal septum epithelia from infected animals revealed mild mononuclear and heterophilic infiltration that was comparable to what was seen in mock-infected animals at both time points, cellular infiltration and moderate goblet cell hyperplasia were observed in the trachea on day 2 and day 4. Similarly, moderate, multifocal infiltration of monocytes, lymphocytes, and heterophils was seen in the bronchioles of one of the infected guinea pigs on day 2 and of both guinea pigs on day 4, with some degree of alveolar thickening in both animals on both days. Mild, multifocal alveolitis was observed in one animal on day 4.

B/Victoria/2/1987 virus is transmitted efficiently to cocaged naïve guinea pigs. Because B/Victoria/2/1987 virus replicated to high titers in inoculated guinea pigs, we hypothesized that the virus would be transmitted to cocaged naïve guinea pigs in a short-range transmission model. Under this experimental setup, transmission via direct and indirect contact, droplet spray, and aerosol are all theoretically possible. Four guinea pigs were first inoculated intranasally with 10^4 PFU of B/Victoria/2/1987 virus and then cocaged with naïve guinea pigs 24 h later. Experiments were con-

ducted at 20°C and 5°C with 20% relative humidity (RH), which was held constant. As expected, the virus replicated to high titers in each of the inoculated guinea pigs, with all but one animal clearing the virus by 8 dpi. Under both environmental conditions tested, virus was detected in all of the naïve cocaged guinea pigs, with all transmission events occurring by day 4 (Fig. 3A and B). In order to confirm these results, guinea pig sera were tested for reactivity to B/Victoria/2/1987 virus by ELISA. All animals seroconverted (Fig. 3C), confirming that short-range transmission had occurred.

B/Victoria/2/1987 virus is transmitted by aerosol/droplet spread and shows similar temperature sensitivity to that of influenza A virus. We hypothesized that B/Victoria/2/1987 virus would be transmitted in a noncontact (aerosol/droplet) model since the virus was transmitted so efficiently under short-range conditions. For these experiments, inoculated and naïve animals were separated by a wire mesh that restricted animal contact.

As in the previous experiment, the virus replicated to high titers in all of the inoculated guinea pigs. At 20°C and 20% RH, all four of the inoculated animals cleared the virus by day 8 postinoculation. Transmission occurred in 2 of the 4 naïve guinea pigs (Fig. 4A). Both transmission events occurred on day 4, and both animals cleared the virus by day 10 of the experiment. An ELISA confirmed these data, as only the two animals that had detectable virus in their nasal passages seroconverted (Fig. 4C).

At 5°C and 20% RH, inoculated guinea pigs shed virus for a longer period: only two of the four animals had cleared the virus

FIG 4 Transmission of B/Victoria/2/1987 virus from inoculated to naïve guinea pigs via aerosol/droplet transmission at 5°C and 20°C. Four animals were inoculated with 10^4 PFU of B/Victoria/2/1987 virus. The following day, each of these animals was placed in a cage with wire siding adjacent to that of a naïve guinea pig; these cages allowed air to flow between cages but restricted direct contact among animals. Nasal washes were performed on days 2, 4, 6, and 8 postinoculation. An additional nasal wash was performed on exposed animals on day 10. Transmission of virus occurred in 50% of naïve guinea pigs that were housed at 20°C (A), while 100% transmission was seen at 5°C (B). Inoculated animals are represented by solid lines; exposed animals are represented by dotted lines. Guinea pig pairs are denoted by the following symbols: squares, pair 1; triangles, pair 2; octagons, pair 3; and diamonds, pair 4. (C) Seroconversion was assessed by ELISA, with serum from a guinea pig infected with an influenza A (H1N1) virus as a negative control.

by day 8. Under these environmental conditions, 100% transmission occurred, and all four naïve guinea pigs were infected by day 6 postinoculation (Fig. 4B). All exposed animals seroconverted to B/Victoria/2/1987 virus (Fig. 4C).

Aerosol/droplet transmission of B/Florida/4/2006 virus is also dependent on temperature. We next wanted to assess whether B/Yamagata-like viruses were similarly able to be transmitted from inoculated to naïve animals in an aerosol/droplet model. As with the previous virus transmission experiment, four guinea pigs were inoculated with B/Florida/4/2006 virus, a B/Yamagata-like virus, and then placed in cages adjacent to those of naïve guinea pigs, at either 5°C or 20°C. RH was again held constant at 20%.

Virus replicated to high titers in the nasal passages of inoculated guinea pigs, and all animals cleared the virus by day 8. As seen with transmission of B/Victoria/2/1987 virus, transmission of virus from inoculated to naïve guinea pigs was more efficient at 5°C than at 20°C. At 20°C, one transmission event occurred on day 4, while another occurred on day 6, resulting in a 50% rate of transmission (Fig. 5A). In contrast, B/Florida/4/2006 virus was transmitted with 100% efficiency at 5°C, with transmission events occurring on day 4 for three animals and on day 6 for the fourth animal (Fig. 5B). An ELISA confirmed these results (Fig. 5C).

Inoculated animals housed at 5°C shed more virus over a longer period than animals housed at 20°C. In both B/Victoria/2/1987 virus transmission experiments, we observed that viral shed-

ding was prolonged in inoculated guinea pigs that were housed at 5°C. Comparing the titers of all 16 intranasally inoculated guinea pigs used in these experiments, nasal wash virus titers were statistically higher in the guinea pigs housed at 5°C on days 4 ($P < 0.01$) and 6 ($P < 0.05$). Differences in day 8 values approached statistical significance ($P = 0.055$) (Fig. 6A). We also assessed whether viral shedding was prolonged in these animals by comparing the last day on which the nasal wash titer was $>10^5$ PFU/ml. This value, termed the “last day of high titer,” was statistically higher ($P < 0.01$) for animals housed at 5°C than for animals housed at 20°C (Fig. 6B). Comparing values for AUC, a calculation that takes both titer and duration of viral shedding into account, those for intranasally inoculated animals housed at 5°C were significantly higher ($P < 0.01$) than those for animals housed at 20°C (Fig. 6C).

Temperature also played a role in viral shedding in the context of B/Florida/4/2006 virus infection. Inoculated guinea pigs housed at 5°C shed statistically more virus on day 4 than those housed at 20°C ($P < 0.001$). The “last day of high titer,” as defined above, was also statistically higher when comparing animals housed under the different environmental conditions. The titer of animals maintained at 20°C fell below 10^5 PFU/ml before day 4, while all of the animals housed at 5°C maintained high titers until after day 4 (Fig. 6E). AUC calculations for animals housed at 5°C were also significantly higher than those for animals housed at 20°C (Fig. 6F), showing that animals housed at the cold temperature shed higher titers of virus for a longer duration.

FIG 5 Transmission of B/Florida/4/2006 virus from inoculated to naïve guinea pigs via aerosol/droplet transmission at 5°C and 20°C. Four animals were inoculated with 10^4 PFU of B/Florida/4/2006 virus. Each of these animals was placed in a cage with wire siding adjacent to a naïve guinea pig on the following day. The cages used allowed air to flow from animal to animal but restricted direct contact. Nasal washes were performed on days 2, 4, 6, and 8 postinoculation. An additional nasal wash was performed on exposed animals on day 10. Transmission of virus occurred in 50% of naïve guinea pigs that were housed at 20°C (A), while 100% transmission was seen at 5°C (B). Inoculated animals are represented by solid lines; exposed animals are represented by dotted lines. Guinea pig pairs are denoted by the following symbols: squares, pair 1; triangles, pair 2; octagons, pair 3; and diamonds, pair 4. (C) Seroconversion was assessed by ELISA, with serum from a guinea pig infected with an influenza A (H1N1) virus as a negative control.

DISCUSSION

Seasonal influenza epidemics normally occur between November and April in the Northern Hemisphere. Conversely, the Southern Hemisphere generally sees a spike in influenza virus-related illness between June and November (48). The seasonality of influenza virus infection is thought to be related, at least in part, to temperature and humidity, as the virus can be transmitted with the highest efficiency under cold, dry conditions (18). Indeed, this reliance has been shown experimentally in the guinea pig model in the context of influenza A virus infection (19, 22). While it has been suggested that temperature may play a role in influenza B virus transmission and rates of infection among humans (48), societal and behavioral confounders may affect the interpretation of epidemiological data such as these. A small animal system with which to study the effects of environmental factors on influenza B virus transmission is therefore warranted. However, due to the limited known host range of influenza B virus, there have been few available animal models with which to study its pathogenicity or transmission and the efficacy of antiviral compounds and vaccines deployed against it.

Here we demonstrate that unadapted human influenza B virus strains are capable of replicating to high titers in the guinea pig. In

addition, we show that representative viruses of both influenza B virus lineages are capable of being transmitted from inoculated to naïve guinea pigs and that, like the case for influenza A virus, the transmission phenotype of these viruses is influenced by temperature. To our knowledge, the guinea pig is the first animal model for which influenza B virus transmission has been described, and as such, it provides a powerful tool for further characterization of influenza B viruses.

In these experiments, cold temperature enhanced the airborne (or aerosol/droplet) transmission efficiency of both B/Victoria/2/1987 and B/Florida/4/2006 viruses. With each virus, 100% of naïve animals became infected at 5°C, while transmission occurred in only 50% of the naïve animals housed at 20°C (Fig. 3 and 4). We hypothesize that this enhanced efficiency was a result of the increased and prolonged shedding of inoculated animals housed at 5°C compared to that for animals housed at the higher temperature (Fig. 6). While these results suggest that transmission of representative viruses from the two main influenza B virus lineages is sensitive to temperature, further investigation is warranted to see if other influenza B virus strains are affected similarly. Investigation is also required to elucidate which factors—whether viral, environmental, or host related—contribute specifically to this

FIG 6 Assessment of viral shedding of inoculated animals housed at 5°C and 20°C. (A) Average viral titers from nasal wash samples collected from B/Victoria/2/87 virus experiments were higher for animals housed at 5°C ($n = 8$) than for those housed at 20°C ($n = 8$) on day 4 and day 6 postinoculation. (B) The last day of high titer occurred later for animals housed at the colder temperature. (C) AUC calculations, which take titer and duration of shedding into account, were elevated for animals maintained at 5°C compared to those housed at 20°C. Similar results were seen following infection with B/Florida/4/2006 virus, as follows. (D) Average viral titers from nasal wash samples were higher for animals housed at 5°C ($n = 4$) than for those housed at 20°C ($n = 4$) on day 4 postinoculation. The last day of high titer also occurred later (E), with higher AUC values for animals housed at the lower temperature (F). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Error bars indicate standard errors of the means for panels A, B, D, and E and standard deviations for panels C and F.

temperature-sensitive transmission phenotype. The viscosity of respiratory secretions, efficiency of mucociliary clearance (36), and potential enhanced stability of the virus at colder temperatures (37), as well as a variety of other factors, could have played a role in the phenotype that was observed. Nevertheless, our data are concordant with the seasonality that is seen with influenza epidemics in temperate climates.

The guinea pig has been a useful model with which to study influenza A virus transmission. It has been shown previously that humidity plays a role in the transmission phenotype of influenza A viruses (19), while fomite transmission is not thought to be an efficient means of viral spread (28). However, the effects of these parameters on influenza B virus transmission are unknown. The guinea pig therefore provides an opportunity to investigate whether these paradigms hold true for influenza B virus while highlighting differences that may exist between influenza A and B viruses.

The circulation of influenza B viruses is intriguing, particularly in comparison to influenza A viruses. In the last century, newly emerging influenza A virus pandemic strains have replaced a prior circulating strain. Viruses of the two influenza B virus lineages, however, though evolutionarily and antigenically distinct, cocirculate in the human population. While B/Victoria-like viruses circulated predominantly in the 1980s, B/Yamagata-like viruses prevailed in the following decade (11, 38), and B/Victoria-like virus circulation became limited geographically to Asia (11, 42). B/Victoria-like viruses began to recirculate globally again during the 2001-2002 influenza season (42) and continue, at present, to cocirculate with B/Yamagata-like viruses. Further complicating this picture, there is often variation in the predominant influenza B virus strain isolated in a given country (7, 52). Influenza B viruses are also known to reassort with one another, sometimes even within a given influenza epidemic (24, 26, 42). The emergence, reappearance, and circulation of influenza B viruses seem to be

distinct from the trends associated with influenza A viruses and warrant further study. Because viruses from both lineages are known to cocirculate, experiments will be designed to study how the immune response to a prior infection with one lineage affects subsequent infection with and transmission of the other. Another intriguing possibility for the guinea pig model is to study the immunological background in which influenza A and B viruses cocirculate and how transmission phenotypes may be affected following co- or sequential infection of viruses of different influenza virus types. Furthermore, the guinea pig model will also be used to study the effects of antivirals and vaccines on influenza B virus transmission.

Influenza B virus is thought to be primarily a human pathogen. While serologic evidence suggests that seals may be a natural host (4, 30, 33), few animals are known to be susceptible to influenza B virus and thus to be adequate models with which to study it. Its limited range suggests that host factors might play a role in limiting its spread. For instance, it has been shown that a critical residue within the M1 protein of influenza B virus, a serine at position 122, confers a pathogenic phenotype in mice when introduced into a normally avirulent influenza B virus strain. Other mutations in the C-terminal domain of M1 are thought to also be critical to pathogenic adaptation of influenza B viruses in mice (25).

NS1 has also been identified as a viral factor that might restrict host range. In animals that are permissive to infection, such as humans and nonhuman primates, influenza B virus NS1 has been shown to bind the interferon-stimulated gene 15 protein (ISG15) and subsequently prevent the ISGylation of target proteins, thus blocking downstream antiviral effects (9, 15, 43, 53). Required amino acids have been identified for this interaction (amino acids D76, K77, and D79); interestingly, these amino acids are not conserved in the predicted guinea pig protein sequence of ISG15 (amino acids K76, D77, and K79). Like in the case for influenza A virus, infection with influenza B virus does not cause overt signs of disease, such as weight loss, in the guinea pig (data not shown). However, influenza B viruses replicate to high titers in the upper respiratory tract of guinea pigs (Fig. 1) and can cause histopathological changes in the guinea pig respiratory tract (Fig. 2). We therefore believe that additional interactions between viral and host proteins must exist in order for influenza B virus to replicate so efficiently in this species. The identification of host restriction factors clearly requires further study, but the ability of unadapted influenza B virus strains to infect guinea pigs suggests that its host range may be broader than initially anticipated and may provide further insight into its adaptation to human hosts in particular.

Due to the limited host range of influenza B viruses, mouse models of infection often require the use of mouse-adapted strains (25, 40) or immunodeficient mice (10, 15, 27). While ferrets have been used routinely as a transmission model for influenza A virus (1), there are no published studies documenting influenza B virus transmission among ferrets. Traditionally, ferrets have been the preferred model with which to study influenza A virus transmission, as these animals display weight loss and fever following virus infection. It has been shown by Kim et al., however, that ferrets infected with B/Victoria-like and B/Yamagata-like viruses display only mild clinical signs and have lower elevations in core temperature than those in animals infected with influenza A viruses (13). This correlates with a reduction in the inflammatory cytokine response as well as a limited ability of influenza B viruses to replicate in the lungs of infected ferrets. Indeed, B/Malaysia/2506/2004 and

B/Shanghai/361/2002 viruses grew to 2- to 3-log lower titers in the lungs than A/New Caledonia/20/1999 and A/Puerto Rico/8/1934 viruses on day 3 postinfection (13). Limited replication of influenza B virus in the respiratory tract could perhaps explain why influenza B virus transmission has yet to be documented for ferrets. In contrast, in the present experiments, B/Malaysia/2506/2004 virus grew to high titers in the nasal passages of infected guinea pigs (Fig. 1), comparable to those seen following influenza A/H1N1 virus infection (8). It is noteworthy, however, that B/Lee/1940 and B/Yamagata/16/1988 viruses did not replicate efficiently in the upper respiratory tract of the guinea pig. We hypothesize that these viruses may have accumulated egg adaptations as a result of repeated passaging over decades. Indeed, all of the recent isolates that were tested grew to high titers in all inoculated animals (Fig. 1), and we were able to observe histological changes in the upper and lower respiratory tracts of guinea pigs infected with the 2006 Florida strain (Fig. 2).

The inefficient replication of influenza B viruses observed by Kim et al. might explain why ferrets have not yet, to our knowledge, been used to study influenza B virus transmission. While there is evidence that cynomolgus macaques can be infected with influenza B viruses and show signs of infection (14), ethical and financial limitations hinder their widespread use for transmission experiments. Therefore, the guinea pig is the first animal model to be described in which this can be studied efficiently.

In conclusion, we present data showing that unadapted influenza B viruses of the B/Victoria-like and B/Yamagata-like lineages can replicate and cause histological changes in the respiratory tract of guinea pigs. Furthermore, we demonstrate that representative viruses from the two main influenza B virus lineages can be transmitted via aerosol/droplet transmission, which is most efficient at low temperatures. The guinea pig therefore provides an opportunity to study influenza B virus transmission and can be used as an important tool in investigating the underlying mechanisms of influenza B virus biology.

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