ruses, because analysis of the HA crystal structure derived from the H7 virus A/Tky/Italy/02 demonstrated specific binding to avian receptor and not human receptor analogues (37). Recent advances in glycan microarray technology allowed us to more closely analyze the fine differences in receptor specificity of viruses between both Eurasian and North American H7 viruses. Here, we used a whole-virus assay that allowed for examination of the binding properties of influenza viruses without the need for generation of recombinant HA. We were particularly interested in the North American H7 viruses, because some of these avian viruses appear to be adapted to the upper respiratory tract of chickens, which have been shown to express more α2–6 SA receptors compared with wild aquatic birds (38); *in vivo* studies have demonstrated that North American lineage LPAI H7N2 viruses replicate to high titer in the upper respiratory tract of chickens and turkeys compared with the gastrointestinal tract (39–41).

All H7 viruses tested replicated to high titer in the upper respiratory tract in inoculated ferrets as shown (13); nevertheless, most isolates tested failed to transmit despite the high titers of virus shed by inoculated animals. Respiratory symptoms such as sneezing and nasal discharge were observed in some ferrets inoculated with each H7 virus (Table 3). However, the frequency and duration of these symptoms in this model more closely resembled those observed in ferrets inoculated with H5N1 viruses, rather than the more pronounced respiratory symptoms observed in ferrets infected with human H3N2 or H1N1 viruses (21, 23). With the exception of NL/219-inoculated ferrets, which exhibit substantial lethargy after infection (13), ferrets inoculated with H7 viruses in this study remained alert and playful for the duration of the experiment, suggesting that frequent interaction between inoculated and contact ferrets is not sufficient for virus transmission to occur. Additionally, the results of this study indicate that increased virus binding to α2–6 SA is not sufficient for transmission of avian influenza viruses to occur, supporting previous studies demonstrating the lack of transmission of an H5N1 virus with an increased α2–6-binding preference (21, 22). Recent studies have highlighted increased complexity of the structural topology among α2–3 and α2–6 SA and suggest that conformational features of the linkage contribute to virus binding and could play a role in virus transmissibility (42). This diversity of SA receptors could in part contribute to the enhanced ability of NL/230 virus to transmit in the ferret model by direct contact compared with NL/219 virus. Although the Eurasian lineage H7N7 viruses analyzed in this study displayed similar receptor-binding properties as measured by glycan array and hemagglutination assay, Munster *et al.* (43) found differential attachment of NL/219 virus and a virus closely related to NL/230 to tissues in the lower respiratory tract of humans. The enhanced transmissibility observed with NL/230 virus in this study compared with NL/219 virus would additionally suggest subtle differences in the receptor-binding properties between these H7N7 viruses that have yet to be identified.

Unlike most subtypes of influenza, infection with H7 influenza viruses frequently results in conjunctivitis in humans and not respiratory disease (6, 7, 9). Unlike the upper respiratory tract in humans, which contains a high distribution of α2–6 SA, corneal, conjunctivial, and lacrimal duct epithelial cells of the human eye express predominantly α2–3 SA (27, 44, 45). Additionally, the sialylated secretions (mucins) of both surfaces differ in their SA content; mucins in the airway epithelium contain α2–3 SA, whereas ocular secreted mucins contain α2–6 SA (46–48). The high α2–3 SA content of the human ocular surface suggests that avian influenza viruses would be well suited to use this surface as a portal of entry. However, although human infection with H7 influenza viruses frequently results in conjunctivitis, documented cases of ocular disease after H5N1 infection are rare (7, 49, 50). The heterogeneity of SA-binding preference observed between H7 influenza virus lineages suggests that, similar to virus transmissibility, ocular tropism is a complex property that cannot be explained by SA receptor binding alone.

We identified a LPAI H7N2 virus, NY/107, which was associated with human respiratory infection and not ocular disease and was effectively transmitted in the ferret model by direct contact (10). Among all H7 viruses analyzed by glycan microarray, NY/107 displayed the most dramatic increase in α2–6 SA binding along with decreased α2–3 SA binding avidity. Strong α2–6 SA binding appears to be an essential component of conferring transmissibility in human influenza viruses, because H1N1 variant viruses exhibiting the classic avian α2–3-binding preference or dual α2–3- and α2–6-binding preference were unable to transmit efficiently (23). These results suggest that a decrease in α2–3 SA binding may also be needed in addition to α2–6 SA-binding avidity. However, despite similar α2–3 and α2–6 SA binding observed by glycan array with Ck/Conn virus, this virus was transmitted only by direct contact in one of three animals. Efficient contact transmission was also not observed with Tky/VA virus, despite this virus sharing 98.4% HA amino acid identity with NY/107 virus (40). Future studies will allow for a better understanding of the genetic determinants responsible for the heightened transmissibility observed with this virus. NY/107 virus, like all H7 viruses tested in this study, did not transmit by respiratory droplets in the ferret model. However, the efficient NY/107 virus transmission observed by direct contact in ferrets has not been observed with HPAI H5N1 viruses (21, 22) and may indicate the capacity of a NY/107-like virus to acquire properties that would confer efficient transmission by respiratory droplets; this underscores the importance of studying virus transmissibility by both routes.

LPAI H7N2 viruses have been acquiring additional basic amino acids at the HA cleavage site since 1994, resulting in a cleavage site that more closely resembles HPAI viruses (51). These viruses are also characterized by a deletion of 8 aa in the HA1 proximal to the receptor-binding site (31); further study will help elucidate whether this deletion contributes to the enhanced α2–6 SA binding observed among these viruses. The classic avian specificity for α2–3-linked SA observed with Rhea/NC could suggest a possible correlation between the acquisition of α2–6 SA binding and the introduction of LPAI H7N2 viruses into the live bird markets of the northeastern U.S. The finding of enhanced α2–6 SA binding of North American H7 viruses underscores the necessity for continued surveillance and study of these viruses as they continue to resemble viruses with pandemic potential.

Materials and Methods

Viruses. Virus stocks were grown in the allantoic cavity of 10-day-old embroynated hens' eggs as described (13). The 50% EID_{50} titer for each virus stock was calculated by the method of Reed and Muench (52), after serial titration in eggs. A/Texas/36/91 (Tx/91) stock was grown on Madin–Darby canine kidney cells containing DMEM, 0.025 M Hepes, 0.3% BSA (Gibco Invitrogen), and *N*-p-tosyl-L-phenylalanine chloromethyl ketone (TPCK)-treated trypsin (Sigma–Aldrich). All experiments with HPAI viruses were conducted under biosafety level 3 containment, including enhancements required by the U.S. Department of Agriculture and the Select Agent Program (53).

Glycan Microarray Analysis. Analysis of the receptor specificity of influenza virus using glycan microarrays was done largely as described (33, 54). Custom arrays for influenza research were produced for the Centers for Disease Control and Prevention on National Health Service-activated glass slides (Schott Nexterion) by using a glycan library provided by the Consortium for Functional Glycomics [www.functionalglycomics.org; see supporting information (SI) Table S1 for a list of glycan structures]. Viruses were inactivated by treatment with β-propiolactone (0.001%) overnight at 4°C with virus inactivation confirmed by two rounds of passage in eggs. Virus preparations were diluted to 1 ml into PBS buffer containing 3% (wt/vol) BSA (PBS-BSA) to HA titers of 256–512. Virus suspensions were applied to slides and the slides were incubated in a closed container and subjected to gentle agitation for 1 h. Unbound virus was washed off by dipping slides sequentially in PBS with 0.05% Tween-20 (PBS-T) and PBS. While still wet, slides were overlaid with corresponding primary antibodies diluted in PBS-BSA, either goat antiserum A/FPV/Rostock/34 (H7N1) (1:500) (for NL/219, NL/230, and Ck/

Conn viruses), ferret anti-A/Canada/444/04 (H7N3) (1:500) (for Can/504 and Can/444 viruses), ferret anti-A/Turkey/VA/4529/02 (H7N2) (1:500) (for Tky/VA virus), ferret anti-A/NY/107/03 (H7N2) (1:500) (for NY/107 virus), chicken anti-A/Rhea/NC/39482/93 (H7N1) (1:500) (for Rhea/NC virus), or sheep anti-A/Vietnam/1203/04 (H5N1) (1:1,000) (for HK/486 virus) (1 h). Slides were washed briefly with PBS-T/PBS as above followed by application of the appropriate secondary antibody conjugates, either anti-ferret-IgG FITC (1:200), anti-goat-IgG FITC (1:200), goat anti-chicken-IgY-FITC (1:200) (Genway Biotechnology), or anti-sheep-IgG-FITC (1:200) in PBS-BSA were subsequently incubated (1 h) followed by PBS-T/PBS washes and a final wash step in deionized water. After the slides were dried in a steam of nitrogen, they were immediately scanned (ProScanArray HT slide scanner with Autoloader, Perkin–Elmer) followed by image analysis with ImaGene 6.1 software (Biodiscovery).

Ferret Transmission Experiments. Male Fitch ferrets, 7–10 months of age (Triple F Farms) and serologically negative by HI assay for currently circulating influenza A H1N1, H3N2, and B viruses were used in this study. Ferrets were housed for the duration of each experiment in a Duo-Flo Bioclean mobile clean room (Lab Products). Ferrets were inoculated with 10^7 EID_{50} of each virus, and nasal washes were collected on indicated days p.i. as described (2). Respiratory droplet and contact transmission experiments were conducted as described (21), with a total of six ferrets used for each experiment.

Hemagglutination Assays. Convalescent sera were collected from all ferrets on days 18–21 p.i./p.c. and tested for H7 specific antibodies by HI by using homologous virus and 1% horse RBCs as described (55). Hemagglutination assays using resialyated turkey RBC were performed as described (56, 57) with minor modifications. Turkey RBC were enzymatically desialyated, followed by resialylation using either α2–6-(*N*)-sialyltransferase (Japan Tobacco) or α2–3-(*N*)-sialyltransferase (Calbiochem). Assays were performed by using both 4 and 8 hemagglutination units of virus yielding identical results.

ACKNOWLEDGMENTS. We thank David Swayne (Southeast Poultry Research Laboratory, U.S. Department of Agriculture, Agricultural Research Service, Athens, GA), Ron Fouchier (Erasmus Medical Center, Rotterdam, The Netherlands), and Yan Li (Canadian Center for Human and Animal Health) for providing some of the viruses used in this study. We also thank Julia Hoffman for early help with the glycan microarray and Anna Crie for assistance with generating the figures. The glycan microarray was produced for the Centers for Disease Control by using a glycan library generously provided by the Consortium for Functional Glycomics funded by National Institute of General Medical Sciences Grant GM62116. J.A.B. received financial support for this work from the Oak Ridge Institute for Science and Education, Oak Ridge, TN. Glycan microarray data presented here will be made available on-line through the Consortium for Functional Glycomics web site upon publication (www.functionalglycomics.org).

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一般的名称	ウロキナーゼ注射剤		研究報告の公表状況	Emerging Infectious Diseases 2008; 14(5): 834-836	公表国 カナダ	
販売名 （企業名）	①ウロキナーゼ 6 万-Wf（ベネシス） ②ウロキナーゼ 12 万-Wf（ベネシス） ③ウロキナーゼ 24 万-Wf（ベネシス）					

研究報告の概要

カナダにおいて、Saffold ウイルスに関連するカルジオウイルス分離株が呼吸器症状を有する 3 名の子供の鼻咽頭吸引物から検出された。Can112051-06 分離株のポリプロテイン配列は、Saffold ウイルスと 91.2%の aa 同一性を有した。しかし、ウイルス表面の EF 及び CD のループは、かなり異なっていた。

ピコルナウイルス科は 9 つの属に分類され、エンテロウイルス、ヘパトウイルス、ライノウイルス、コブウイルス及びパレコウイルスはヒトに感染するが、アフトウイルス、エルボウイルス、テスコウイルス及びカルジオウイルスは動物の病原体である。カルジオウイルス属は、タイラーウイルスと脳心筋炎ウイルス（EMCVs）の 2 つの種に分けられる。ラットやマウスは EMCVs の自然宿主であるが、これらのカルジオウイルスはこれまで、ブタ、げっ歯類、ゾウ、マカク及びヒトに感染することがわかっている。最近、Saffold ウイルス（SAF-V）と暫定的に名付けられた新規カルジオウイルスが、発熱した 8 歳の女子の排便サンプルから分離された。このウイルスは、新規のカルジオウイルスの種と考えられ、知られている他のカルジオウイルスよりも遺伝的にタイラー様ウイルスに関連していた。
SAF-V と、今回の研究で示した SAF-V に関連するカナダの株が、新種のヒトカルジオウイルスの種と分類されるべきか、あるいはタイラーウイルス種の中の新しいクレードとして分類されるべきかについて、今後決定される必要がある。

使用上の注意記載状況・その他参考事項等

感染症に関連する記載はない。

報告企業の意見	今後の対応
新規の Saffold ウイルスに関連するカルジオウイルス分離株が呼吸器症状を有する 3 名の子供の鼻咽頭吸引物から検出されたとの報告である。 カルジオウイルスは、ピコルナウイルス科に属する直径 22～30nm のエンベロープを有しない RNA ウイルスである。血漿分画製剤からのカルジオウイルス感染に関する報告は、入手していない。 万一、添加剤のアルブミンの原料血漿にカルジオウイルスが混入したとしても、EMCおよびCPVをモデルウイルスとしたウイルスバリデーション試験成績から、製造工程において十分に不活化・除去されると考えている。	新規カルジオウイルスに関する追加情報の入手に努める。

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