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Address for correspondence: William E. Sander, Department of Veterinary Clinical Medicine, University of Illinois at Urbana–Champaign, 1008 W Hazelwood Dr, Urbana, IL 61801-3028, USA; email: wsander@illinois.edu

Reassortant Influenza A(H1N1)pdm09 Virus in Elderly Woman, Denmark, January 2021

Jakob N. Nissen, Sophie J. George, Charlotte K. Hjulsager, Jesper S. Krog, Xiaohui C. Nielsen, Tina V. Madsen, Klara M. Andersen, Tyra G. Krause, Lasse S. Vestergaard, Lars E. Larsen, Ramona Trebbien

Author affiliations: Statens Serum Institut, Copenhagen, Denmark (J.N. Nissen, C.K. Hjulsager, J.S. Krog, K.M. Andersen, T.G. Kause, L.S. Vestergaard, R. Trebbien); University of Copenhagen, Copenhagen (S.J. George, L.E. Larsen); Zealand University Hospital, Koege, Denmark (X.C. Nielsen, T.V. Madsen)

DOI: https://doi.org/10.3201/eid2712.211361

A case of human infection with influenza A(H1N1)pdm09 virus containing a nonstructural gene highly similar to Eurasian avian-like H1Nx swine influenza virus was detected in Denmark in January 2021. We describe the clinical case and report testing results of the genetic and antigenic characterizations of the virus.

Human infection with swine influenza A virus (IAV) had not previously been detected in Denmark, but sporadic cases have been reported from other countries (*1*). We report the identification of a case of zoonotic swine influenza infection in Denmark during a low-activity influenza season.

The variant IAV was detected by the National Influenza Center at Statens Serum Institut (Copenhagen, Denmark), as part of routine surveillance. A sputum sample was collected on January 21, 2021, in Zealand, Denmark, from a female patient in her 70s with various concurrent conditions, including a chronic respiratory disease, who was admitted to hospital after 2 days of moderate influenza-like symptoms: fever (39°C), coughing, sore throat, and difficulty breathing. The patient sample was positive for IAV in analyses at the local hospital microbiology laboratory; remaining sample material was submitted to the National Influenza Center, which confirmed it positive for influenza A(H1N1)pdm09 (Appendix, https://wwwnc.cdc.gov/EID/article/27/12/21-1361-App1.pdf).

We performed whole genome sequencing on the virus (*2*), and named it A/Denmark/1/2021 (vH1N1), and submitted to GISAID (https://www.gisaid.org; accession no. EPI_ISL_909652). BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and phylogenetic analyses revealed that all segments except the nonstructural gene belonged to influenza A(H1N1)pdm09 clade 1A3.3.2 (*3*), which is most similar (97%–98% nt identity) to viruses collected from swine in France and Germany in 2014 and 2015 (Table; Figure). The nonstructural gene was most similar (95%) to Eurasian avian-like H1Nx swine viruses of clade 1C. No segments had a near-exact match to sequences in GenBank or GISAID, and all were distinct from the seasonal vaccine strain, A/Guangdong-Maonan/SWL1536/2019 (Table).

Because of the suspected swine origin of the case virus, we used whole-genome sequencing to retrospectively analyze 68 IAVs with a hemagglutinin (HA) gene belonging to clade 1A.3.3.2 sampled from swine herds in Denmark during 2020–2021. Nine of the samples, collected April 2020–January 2021 from ≥7 different herds in different parts of Denmark, including Zealand, contained the same

Table. Percentage identity similarity between gene and protein segments of influenza virus isolate A/Denmark/1/2021 (vH1N1) from a patient in Denmark and reference viruses from GISAID*

A/Denmark/1/2021 (vH1N1) segment	Identity, %			
	A/swine/Luedinghausen/ 21728/2015†	A/California/07/2009‡	A/Guangdong-Maonan/SWL1536/2019¶	A/swine/Denmark/ 3797–4/2020§
Amino acid				
PB2	98.7	97.5	97.6	100
PB1	99.5	99.3	98.7	99.9
PA	98.9	98.0	98.3	99.6
PA-X	98.7	97.4	97.0	99.6
HA	97.3	92.0	91.9	99.3
NP	99.0	99.0	98.2	100
NA	97.9	95.1	91.9	99.8
M1	98.8	98.4	97.6	100
M2	96.9	96.9	93.8	100
NS1	76.5	77.4	74.7	99.5
NEP	85.1	86.0	85.1	99.2
Nucleotide				
PB2	98.0	96.1	94.7	99.8
PB1	96.8	95.9	93.8	99.2
PA	98.0	96.7	95.4	99.4
HA	97.3	94.4	93.0	99.4
NP	97.4	96.4	94.5	99.4
NA	97.4	96.1	93.7	99.5
MP	97.8	97.4	95.9	99.9
NS	80.2	80.3	80.3	99.8

*GISIAD, https://www.gisaid.org. PB1/PB2, polymerase basic protein 1/2; PA, polymerase acidic protein; HA, hemagglutinin; NP, nucleoprotein; NA, neuraminidase; MP/M1/M2, matrix protein 1/2, NS/NS1; nonstructural protein; NEP, nuclear export protein
†GISAID accession no. EPI_ISL_504870.
‡GISAID accession no EPI_ISL_227813.
¶GISAID accession no. EPI_ISL_377080.
§GISAID accession no. EPI_ISL_1673668.

gene constellation as the case virus (98.9%–99.4% nt identity). This finding suggests that the virus from the human case originated from swine in Denmark.

The patient and her husband reside in the countryside, <2 km from a medium-sized farm with finisher pigs. Because of coronavirus disease pandemic restrictions, she had not been in close contact with other persons or been close to the pig farm. Both the patient and her husband, who had no signs of illness, were vaccinated against seasonal influenza in October or November 2020. European General Data Protection Regulation (https://gdpr.eu) restrictions on reporting personally identifiable information prevent revealing additional information about the patient or the farm.

Veterinary authorities in Denmark collected nose swab samples from 68 pigs at the neighboring farm on February 1, 2021, according to standard procedures. All samples tested negative by PCR for IAV. Because of the high prevalence of influenza-positive herds in Denmark, we could not be confident potential seropositive swine were infected by the virus in question, so we did not take blood samples. However, we therefore could not exclude previous virus circulation in the herd, because swabs were taken 11 days after virus detection in the patient. According to the Danish Meteorological Institute, the patient's residence was downwind of the pig herd most days preceding clinical symptoms.

Most of the case virus genes were derived from influenza A(H1N1)pdm09, which has been circulating in the human population of Denmark since 2009. However, the HA gene is different from that of the strains currently circulating (*4*), and it is therefore difficult to predict the level of immunity in the human population against this virus. Antigenic characterization (*5*) showed no or very poor cross-reactivity to all reference antiserum used for analysis (Appendix Table 1), and the HA gene contained several more mutations at antigenic sites compared with the seasonal vaccine strain (Appendix Figure). Therefore, vaccine effectiveness of the 2020–2021 seasonal influenza vaccine against the variant virus has been assessed as low.

Neuraminidase inhibition tests showed no reduction of oseltamivir or zanamivir inhibition, and the viral genome contains no known antiviral mutations except the V27A mutation in the M2 gene, known from most other H1N1 viruses circulating in human and swine (*6*,*7*). We identified no amino acid changes presumed to be related to increased risk of human infection (*8*), but further in vitro and in vivo analyses are planned to explore this possibility.

Because national coronavirus disease pandemic restrictions limited interpersonal contact, there

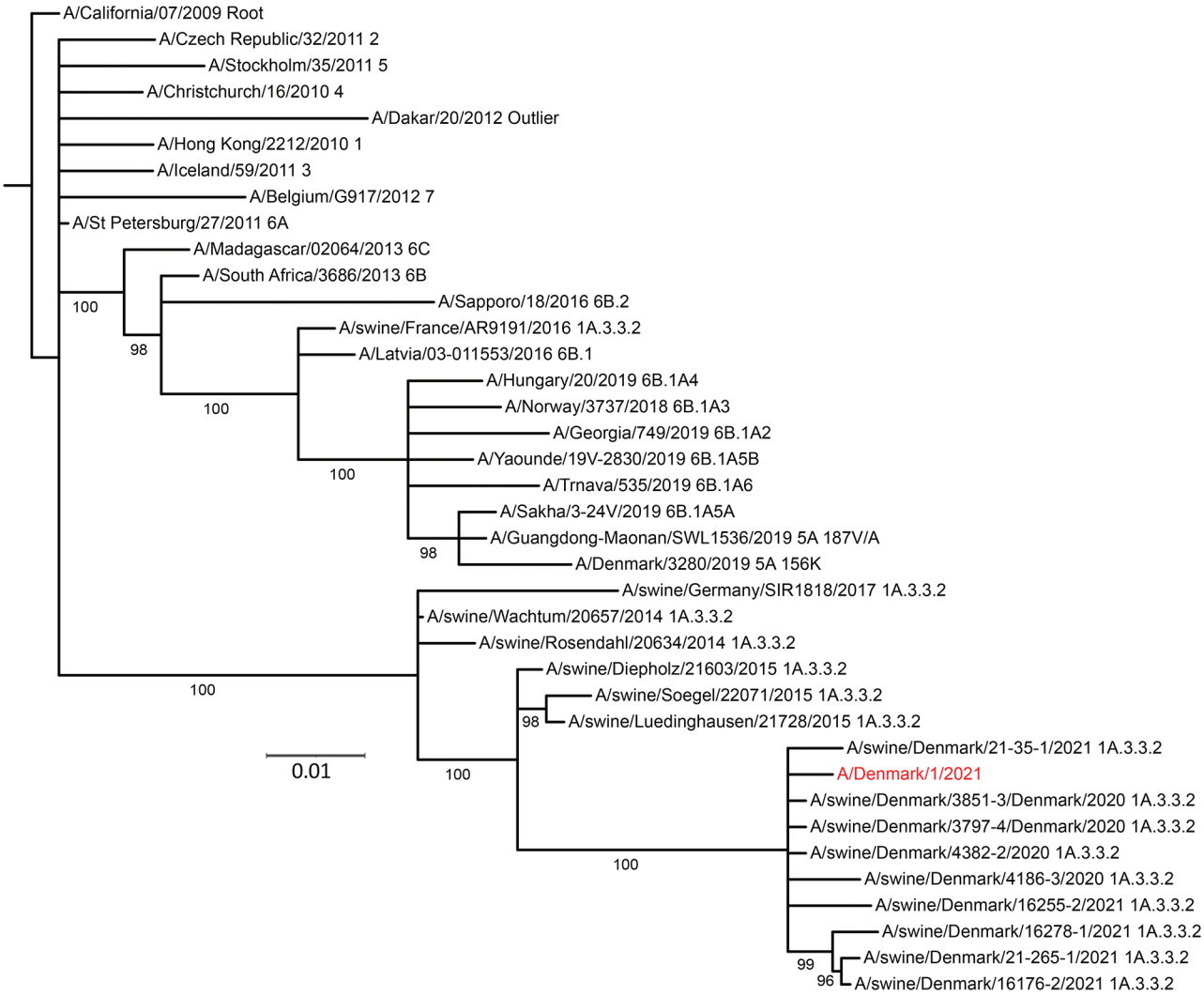

Figure. Maximum-likelihood phylogenetic tree of the hemagglutinin gene of influenza virus isolate A/Denmark/1/2021 (vH1N1) from a patient in Denmark (red) and reference viruses. The tree includes closest BLAST matches (https://blast.ncbi.nlm.nih.gov/Blast.cgi), the Denmark swine influenza virus with highest similarity to the case variant virus A/Denmark/1/2021 (indicated in red), and human seasonal reference viruses and is rooted on A/California/07/2009. Leaves are labeled by isolate name and clade designation. Branch labels indicate UFBoot2 bootstrap values. All uncertain branches (bootstrap <95%) have been removed. Scale bar indicates nucleotide substitutions per site.

were only 46 confirmed influenza cases in Denmark during the 2020–2021 season, and transmission of the variant virus was considered negligible. The Danish Patient Authority did not identify any person-to-person swine influenza transmission, and no further public health response measures were enacted.

The effects of the most recent swine influenza pandemic and the extensive diversity and reassortment in swine influenza viruses indicate the obvious zoonotic potential of these viruses (*9*,*10*). Therefore, more attention should be given to routine detection and control of swine influenza viruses.

Acknowledgments

We thank laboratory technicians Mille Weissman Poulsen, Carina Bøgh Folsing, Jesper Rønn, Sari Mia Dose, and Sophia Rasmussen for technical assistance in the laboratory.

This work was conducted as part of the national influenza surveillance in Denmark, which is funded by the government.

About the Author

Dr. Nissen is a postdoctoral researcher at the National Influenza Center, Statens Serum Institut, Copenhagen,

Denmark. With a background in genomics and bioinformatics, he focuses on bioinformatic tool development and the prediction of the zoonotic potential of influenza viruses.

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Address for correspondence: Ramona Trebbien, Statens Serum Institut, Artillerivej 5, 2300 Copenhagen S, Denmark; email: ratr@ssi.dk

Correction: Vol. 27, No. 10

The name of author Xiaohui Wang was misspelled in *Emergomyces orientalis* Emergomycosis Diagnosed by Metagenomic Next-Generation Sequencing (D. He et al.). The article has been corrected online (https://wwwnc.cdc.gov/eid/article/27/10/21-0769_article).

Article DOI: https://doi.org/10.3201/eid2712.211361

Reassortant Influenza A(H1N1)pdm09 Virus in Elderly Woman, Denmark, January 2021

Appendix

Methods for Virus Detection and Analysis

We collected sputum samples and analyzed them at the local hospital microbiology laboratory with the SARS-CoV-2 Flu (A+B) & RSV array (CerTest Biotec, https://www.certest.es) on the BD MAX System (Becton Dickinson, https://www.bd.com) and the Xpert Xpress Flu/RSV assay on the GeneXpert XVI system (Cepheid, https://www.cepheid.com). Subsequently, the National Influenza Center analyzed remaining sample materials using in-house real-time reverse transcription PCR to detect the matrix-, H1pdm09-, and N1pdm09-gene segments. For whole genome sequencing, a one-tube RT-PCR approach was used (*1*), and libraries for sequencing on the Miseq platform (Illumina, https://www.illumina.com) were prepared using the Nextera XT DNA preparation kit (Illumina) (*2*).

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Appendix Table 1. Results from antigenic characterization using hemagglutination inhibition assay and microneutralization assay*

Reference virus	A/Guangdong-Maonan/SW1636/2019	A/Victoria/2570/19	A/Wisconsin/588/19	A/Denmark/3280/29	A/Michigan/45/15	A/California/07/09	A/Brisbane/02/2018
Hemagglutinin inhibition test results							
A/Guangdong-Maonan/SW1636/2019	2560						
A/Victoria/2570/19		640					
A/Wisconsin/588/19			1280				
A/Denmark/3280/29				<2560			
A/Michigan/45/15					640		
A/California/07/09						640	
A/Brisbane/02/2018							960
A/Denmark/1/2021	<20	<20	<20	<20	30	<20	<20
Microneutralization results							
A/Michigan/45/15					640	120	
A/California/07/09					40	240	
A/Guangdong-Maonan/SW1636/2019	7680					1280	
A/California/07/09	640					1920	
A/Denmark/1/2021	<20	20	20	20	<20	30	30

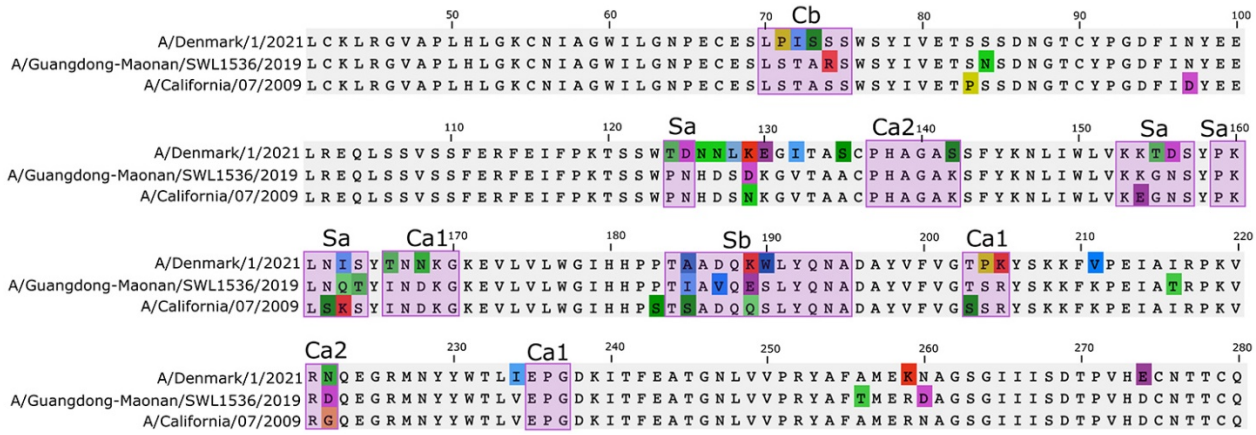
*The case variant virus was tested against a panel of reference ferret antisera of A/H1N1pdm09 viruses provided by WHO CC, Francis Crick Institute, UK. The cross-testing of viruses and antisera are indicated with the average titer-value from duplicates. A titer below 20 is considered as no reaction. Thus, in both tests there was no cross-reactivity to the A(H1N1)pdm09 vaccine virus but to some of the other A(H1N1)pdm09 viruses at low levels.

Appendix Table 2. We gratefully acknowledge the authors, originating and submitting laboratories of the sequences from GISAID's EpiFlu Database on which this research is based. All submitters of data may be contacted directly via www.gisaid.org

Segment ID*	Country	Collection date	Isolate-ID	Isolate name	Originating Lab	Submitting Lab	Authors
EPI1785107	Germany	2015 Aug 26	EPI_ISL_504872	A/swine/Soegel/22071/2015		Import from public-domain	Duerrwald, R.; Groth, M.; Krumbholz, A.; Lange, J.; Philipps, A.; Zell, R.
EPI1785091	Germany	2015 May 21	EPI_ISL_504870	A/swine/Luedinghausen/21728/2015		Import from public-domain	Duerrwald, R.; Groth, M.; Krumbholz, A.; Lange, J.; Philipps, A.; Zell, R.
EPI1785075	Germany	2015 Apr 15	EPI_ISL_504868	A/swine/Diepholz/21603/2015		Import from public-domain	Duerrwald, R.; Groth, M.; Krumbholz, A.; Lange, J.; Philipps, A.; Zell, R.
EPI1784875	Germany	2014 Aug 10	EPI_ISL_504843	A/swine/Wachtum/20657/2014		Import from public-domain	Duerrwald, R.; Groth, M.; Krumbholz, A.; Lange, J.; Philipps, A.; Zell, R.
EPI1784859	Germany	2014 Sep 16	EPI_ISL_504841	A/swine/Rosendahl/20634/2014		Import from public-domain	Duerrwald, R.; Groth, M.; Krumbholz, A.; Lange, J.; Philipps, A.; Zell, R.
EPI1201916	Germany	2017 Mar 10	EPI_ISL_304243	A/swine/Germany/SIR1818/2017	Friedrich-Loeffler-Institut	Friedrich-Loeffler-Institut	Harder, Timm C.; Henritzi, Dinah
EPI1080448	France	2016 Nov 14	EPI_ISL_281884	A/swine/France/AR9191/2016		Friedrich-Loeffler-Institut	Henritzi, Dinah; Harder, Timm C.
EPI1365955	Hungary	2018 Dec 17	EPI_ISL_340478	A/Hungary/20/2019	National Public Health Institute	Hungarian National Center of Epidemiology	
EPI1582934	Cameroon	2019 Apr 16	EPI_ISL_388855	A/Yaounde/19V-2830/2019	Centre Pasteur du Cameroun	Crick Worldwide Influenza Centre	
EPI1542971	Georgia	2019 Apr 08	EPI_ISL_377238	A/Georgia/749/2019	National Centre for Disease Control and Public Health	Crick Worldwide Influenza Centre	
EPI748982	Latvia	2016 Feb 29	EPI_ISL_219670	A/Latvia/03–011553/2016	State Agency, Infectology Center of Latvia	Crick Worldwide Influenza Centre	
EPI1543082	Slovakia	2019 Apr 10	EPI_ISL_377294	A/Trnava/535/2019	National Public Health Institute of Slovakia	Crick Worldwide Influenza Centre	
EPI1575087	Russian Federation	2019 Apr 24	EPI_ISL_387098	A/Sakha/3–24V/2019	State Research Center of Virology and Biotechnology	Crick Worldwide Influenza Centre	
EPI1161425	United States	2009 Apr 09	EPI_ISL_227813	A/California/07/2009		Import from public-domain	Tan, G.; Pickett, B.; Fedorova, N.; Amedeo, P.; Isom, R.; Hu, L.; Christensen, J.; Miller, J.; Durbin, A.; Arumemi, F.; Williams, T.; Bao, Y.; Sanders, R.; Zhdanov, S.; Kiryutin, B.; Lipman, D.J.; Tatusova, T.; Hatcher, E.; Wang, J.
EPI1542570	China	2019 Jun 17	EPI_ISL_377080	A/Guangdong-Maonan/SWL1536/2019	WHO Chinese National Influenza Center	WHO Chinese National Influenza Center	Xiaoxu, Zeng; Xiyan, Li; Weijuan, Huang; Lei, Yang; Dayan, Wang
EPI466580	Madagascar	2013 May 24	EPI_ISL_145424	A/Madagascar/02064/2013	Institut Pasteur de Madagascar	National Institute for Medical Research	

Segment ID*	Country	Collection date	Isolate-ID	Isolate name	Originating Lab	Submitting Lab	Authors
EPI417118	Belgium	2012 Dec 07	EPI_ISL_134397	A/Belgium/G917/2012	Scientific Institute of Public Health	National Institute for Medical Research	
EPI346697	Iceland	2011 Mar 24	EPI_ISL_99924	A/Iceland/59/2011	Landspitali - University Hospital	National Institute for Medical Research	
EPI417122	Senegal	2012 Dec 09	EPI_ISL_134399	A/Dakar/20/2012	Institut Pasteur de Dakar	National Institute for Medical Research	
EPI770076	Japan	2016 Feb 01	EPI_ISL_223792	A/SAPPORO/18/2016	Sapporo City Institute of Public Health	National Institute of Infectious Diseases	Takashita, Emi; Fujisaki, Seiichiro; Shirakura, Masayuki; Watanabe, Shinji; Odagiri, Takato
EPI347564	Sweden	2011 Nov 22	EPI_ISL_100460	A/Stockholm/35/2011		Public Health Agency of Sweden	
EPI705858		2016 Feb 09	EPI_ISL_210175	A/Christchurch/16/2010 NIB-74xp (13/202)	National Institute for Biologic Standards and Control (NIBSC)	National Institute for Biologic Standards and Control	Nicolson, Carolyn
EPI279895	Hong Kong (SAR)	2010 Jul 16	EPI_ISL_79623	A/Hong Kong/2212/2010	Government Virus Unit	National Institute for Medical Research	
EPI319447	Czech Republic	2011 Jan 18	EPI_ISL_90718	A/Czech Republic/32/2011	National Institute of Public Health	National Institute for Medical Research	
EPI319527	Russian Federation	2011 Feb 14	EPI_ISL_90760	A/St. Petersburg/27/2011	WHO National Influenza Centre Russian Federation	National Institute for Medical Research	
EPI466630	South Africa	2013 Jun 10	EPI_ISL_145449	A/South Africa/3686/2013	National Institute for Communicable Disease	National Institute for Medical Research	
EPI1393451	Norway	2018 Nov 27	EPI_ISL_347404	A/Norway/3737/2018	WHO National Influenza Centre	Crick Worldwide Influenza Centre	

*All segments are hemagglutinin.



Appendix Figure. Alignment of HA amino acid sequences of case variant virus A/Denmark/1/2021, A/California/07/2009, and seasonal vaccine strain A/Guangdong-Maonan/SWL1536/2019. The alignment is shown with H1 numbering starting after the signal peptide, and the antigenic sites Ca1, Ca2, Cb, Sa, Sb, as defined by Brownlee and Fodor (*3*), are indicated with shaded boxes. Mutations relative to each other have been highlighted.