11). In human case-patients, the first-choice therapy is to remove the worms mechanically by flushing the conjunctival sac with sterile physiologic saline under local anesthesia (Appendix reference *12*).

From a therapeutic and epidemiologic standpoint, it is important to differentiate between infectious and allergic conjunctivitis. Furthermore, diagnosis can be difficult because immature larvae can hide in the excretory ducts of the lacrimal glands (*7*). Our findings indicate the need for education and raised awareness about this infection especially for ophthalmologists. Early and adequate diagnosis can help to prevent complications such as corneal ulceration.

About the Author

Ms. Juhász is a biologist specializing in clinical microbiology in the Department of Medical Microbiology, University of Szeged, Szeged, Hungary. Her primary research interests are human viral, bacterial, and parasitic infections, focusing on respiratory tract pathogens.

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Severe Human Case of Zoonotic Infection with Swine-Origin Influenza A Virus, Denmark, 2021

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DOI: https://doi.org/10.3201/eid2812.220935

During routine surveillance at the National Influenza Center, Denmark, we detected a zoonotic swine influenza A virus in a patient who became severely ill. We describe the clinical picture and the genetic characterization of this variant virus, which is distinct from another variant found previously in Denmark.

Human infections with swine influenza A viruses (IAVs) are sporadically reported (*1*–*4*). Increased surveillance has revealed substantial swine IAV circulation within pig herds and frequent reassortment with human seasonal IAVs (*5*). Despite no sustained human-to-human transmission of variant IAV cases since the 2009 influenza A(H1N1) pandemic, the zoonotic potential is of concern. We report a case of human infection with a swine-origin IAV that resulted in severe illness in a younger, otherwise healthy person employed at a swine slaughterhouse in Denmark. This case was detected 10 months after our previously reported case (*4*). The patient provided informed consent for publication of this case report.

On November 24, 2021, a person of ≈50 years of age was hospitalized after acute onset of illness characterized by dizziness on the night of November 23, 2021, followed by chest pain, pain radiating toward the left arm, diarrhea, and malaise that developed the next morning, but no fever. The patient called for emergency medical assistance, which arrived shortly. During ambulance transportation and at hospital arrival, the patient experienced repeated convulsions and was admitted to the intensive care unit and put on mechanical ventilation to manage seizures and associated reduced oxygen level. Extensive clinical examination, such as laboratory investigations (i.e., biochemical, microbiological, and immunological assays), multiorgan radiological examinations, and electroencephalography (Appendix 1, https://wwwnc.cdc.gov/EID/article/28/12/22-0935-App1.pdf), identified no cardiovascular, renal,

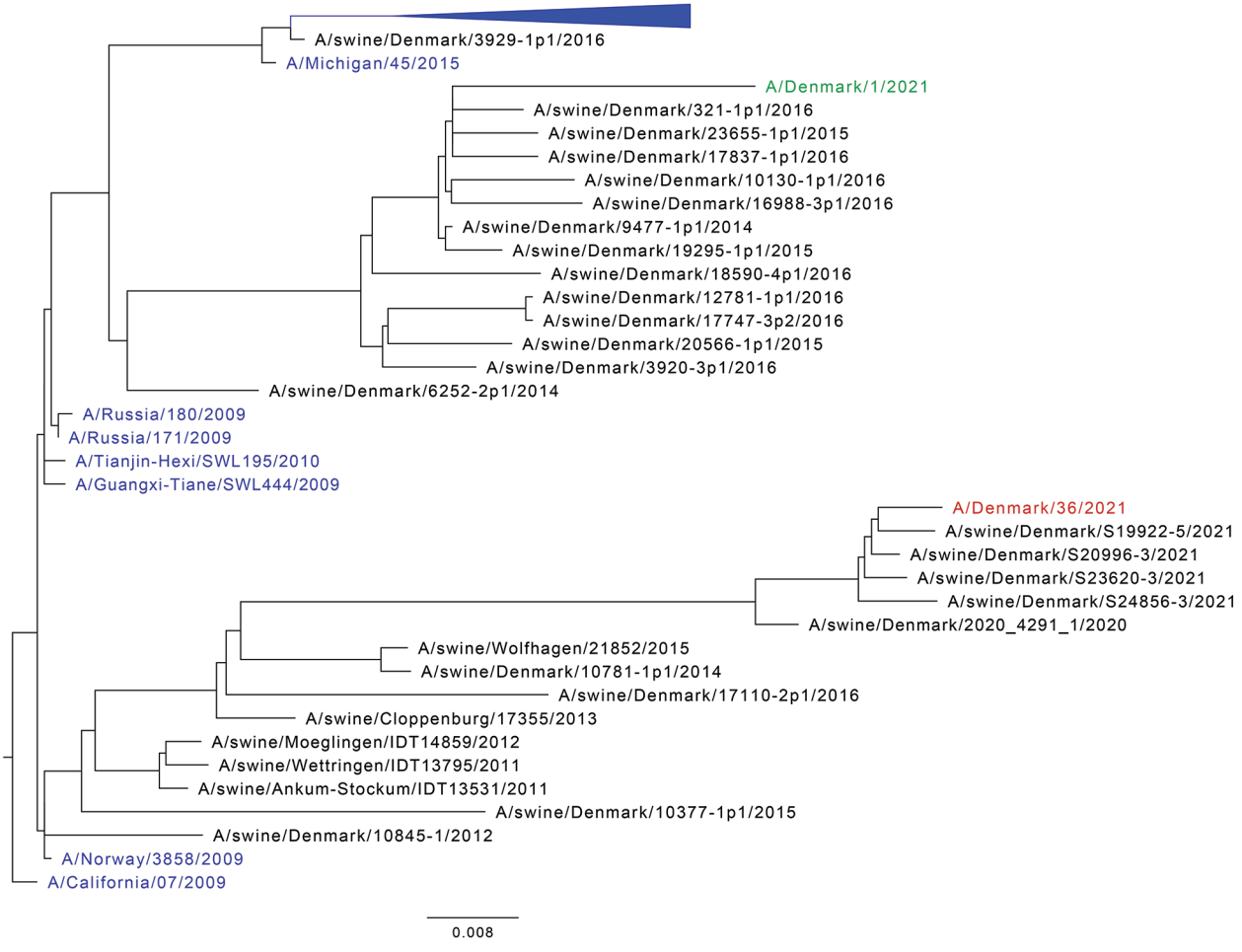

Figure. Maximum-likelihood phylogenetic tree of the hemagglutinin gene of influenza A virus from a patient in Denmark (A/Denmark/36/2021), the seasonal vaccine strain, and closely related strains. The tree includes the case variant virus A/Denmark/36/2021 (red), the 10 closest BLAST matches (https://blast.ncbi.nlm.nih.gov/Blast.cgi), the previously reported Denmark variant virus A/Denmark/1/2021 (green), human seasonal reference viruses with >85% nucleotide identity to A/Denmark/36/2021 (Appendix 2 Table, https://wwwnc.cdc.gov/EID/article/28/12/22-0935-App2.xlsx), and representative viruses from the passive surveillance program of influenza viruses in pigs from Denmark. The tree is rooted on A/California/07/2009. Human IAV sequences are shown in blue, and most seasonal reference viruses have been collapsed. Scale bar indicates nucleotide substitutions per site.

Table. Percentage nucleotide and amino acid identities between influenza A virus from a patient in Denmark (A/Denmark/36/2021), the seasonal vaccine strain, and closely related strains*

Gene	A/Victoria/2570/2019	A/swine/Denmark/S19922-5/2021	A/swine/Denmark/24856-3/2021	A/swine/Denmark/S22282-5/2021	A/Denmark/1/2021
Pairwise nucleotide identity to A/Denmark/36/2021, %					
PB2	94.8	99.4	98.9	99.4	96.1
PB1	94.7	99.5	98.9	99.3	93.6
PA	95.2	99.8	99.3	99.6	96.8
HA	90.7	99.0	98.7	72.6	89.9
NP	95.3	99.7	98.9	99.4	96.4
NA	86.9	99.3	98.7	n/a	87.0
MP	95.2	99.6	99.6	92.6	95.3
NS	79.7	99.9	99.1	99.6	92.8
Pairwise amino acid identity to A/Denmark/36/2021, %					
PB2	97.2	99.6	99.5	99.6	97.9
PB1	97.6	99.9	99.5	99.9	97.9
PA	97.6	99.9	99.3	99.4	98.3
PA-X	96.6	100	100	100	98.7
HA	90.3	98.8	98.6	75.8	88.5
NP	98.2	100	99.6	99.8	98.4
NA	85.1	98.9	97.4	n/a	86.1
M1	97.6	100	100	97.6	97.6
M2	94.9	100	100	90.7	93.8
NS1	72.4	99.6	98.3	99.1	91.7
NEP	85.1	100	100	100	95

*Seasonal vaccine strain, A/Victoria/2570/2019 (GISAID isolate no. EPI_ISL_517733; Appendix 2, https://wwwnc.cdc.gov/EID/article/28/12/22-0935-App2.xlsx); strains from passive surveillance Denmark: A/swine/Denmark/S19922–5/2021 (GenBank accession nos. ON716251–8), A/swine/Denmark/24856-3/2021 (accession nos. ON716275–82), A/Denmark/S22282-5/2021 (accession nos. ON716267–74); and another recent variant case found in Denmark (A/Denmark/1/2021; GISAID isolate no. EPI_ISL_909652) (*4*). The A/swine/Denmark/S22282-5/2021 is of the H1N2 subtype and therefore no percentage similarity is reported to the neuraminidase gene segment and protein of this strain. HA, hemagglutinin; MP/M1/M2, matrix protein 1/2; NA, neuraminidase; n/a, not applicable; NEP, nuclear export protein; NP, nucleoprotein; NS/NS1, nonstructural protein; PA, polymerase acidic protein; PB1/2, polymerase basic protein 1/2.

neurologic, or other diseases that could explain the sudden severe illness. However, a tracheal sample collected and analyzed at the local microbiology laboratory was found positive for IAV (Appendix 1). No other microbiological agents were detected, including SARS-CoV-2 or other respiratory viruses, and the patient showed no signs of pneumonia. The patient received antiviral medication (oseltamivir) and various supportive treatments, and over the next 2 days the clinical condition improved; the patient was soon after discharged from the hospital.

The remaining sample material was submitted to the Danish National Influenza Center as part of routine influenza surveillance. The sample was confirmed positive for the pandemic H1N1 strain and was further analyzed by whole-genome sequencing (Appendix 1). Consensus sequences for the virus named A/Denmark/36/2021 were uploaded to GISAID (https://www.gisaid.org; isolate no. EPI_ISL_8786194). WGS confirmed the H1N1 subtype; however, the virus had closer similarity to swine IAVs (Figure) than to other human strains. BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) searches revealed no close matches to IAV sequences in GenBank or GISAID, but comparison to in-house sequences from the passive surveillance of influenza viruses in pigs from Denmark revealed close similarity to 2021 swine IAVs (Table). Phylogenetic analyses showed that most gene segments were related to the pandemic H1N1 subtype (clade 1A3.3.2), whereas the neuraminidase and nonstructural segments belonged to the clade 1C Eurasian avian-like swine influenza A(H1N1) (Figure; Appendix 1 Figures 1–7). In contrast, another variant virus found recently in Denmark had a clade 1C nonstructural segment, whereas the 7 other gene segments were related to clade 1A3.3.2 pandemic H1N1 viruses (*4*).

In-depth interviews with the patient revealed occupational exposure to swine in a pig slaughterhouse in Denmark, which appears the most likely place of infection. The patient handled live pigs, carcasses, and meat during the slaughtering process while wearing protective equipment including gloves and gown but no face mask. The patient was previously healthy, had no underlying diseases or immune deficiencies, and had received the recommended quadrivalent seasonal influenza vaccine in October 2021.

No other cases of influenza had been reported at the patient's workplace or among close contacts. In the 2021–22 influenza season, 16,160 cases of influenza A virus occurred among 244,184 tested samples in Denmark; the H3N2 subtype was dominant. No other human cases of swine-origin influenza virus were detected during this period. Genetic analyses and antigenic characterization of the virus (Appendix 1 Table 1, Figure 8) showed

several genetic and antigenic differences and suggested poor reactivity to the contemporary human seasonal influenza vaccine.

This reported case is considered independent of the previously reported variant infection in Denmark (*4*), because the 2 viruses are genetically distinct (Table). The symptoms were also different; the earlier case was in an elderly patient with comorbidities who experienced classical influenza-like illness, but in this case, a previously healthy adult of younger age experienced unusual severe and sudden illness. Influenza-associated convulsions in adults are rare (*6*) and mostly accompanied by fever or encephalitis, which was not observed in this patient.

The identification of variant IAVs emphasizes the zoonotic potential of these strains and highlights the importance of continued monitoring of both human and swine IAVs. The reported case suggests a need for focusing on early registration of swine exposure for humans with influenza-like illness, as well as increased measures to reduce the swine IAV exposure risk for people with occupational contact with swine.

Acknowledgments

We thank the laboratory technicians from the National Influenza Center at Statens Serum Institute for technical assistance in the laboratory. We also thank the patient for collaboration through the interviews and for allowing publication of the case.

This work has been conducted as part of the national influenza surveillance in Denmark, which is funded by the government, and as part of the FluZooMark project, funded by the Novo Nordisk Foundation (grant no. NNF19OC0056326).

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Autochthonous *Angiostrongylus cantonensis* Lungworms in Urban Rats, Valencia, Spain, 2021

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DOI: https://doi.org/10.3201/eid2812.220418

[1]These first authors contributed equally to this article.

Article DOI: https://doi.org/10.3201/eid2812.220935

Severe Human Case of Zoonotic Infection with Swine-Origin Influenza A Virus, Denmark, 2021

Appendix

Methods for Virus Detection and Analysis

A tracheal sample was collected and analyzed at the local hospital microbiology laboratory using Cepheid Xpert Xpress SARS-CoV-2_Flu_RSV assay (Cepheid, Sunnyvale, CA). The sample had a cp-value of 23.9 for assay target FluA 1 and a cp-value of 26.3 for the assay target FluA 2. The sample was negative for Influenza B, SARS-CoV-2 and RSV.

At the National Influenza Center at Statens Serum Institut, Copenhagen, Denmark, the remaining sample material was analyzed by in-house real-time RT-PCR which detects the matrix- and H1pdm09-gene segments. Here, the sample was confirmed influenza A H1pdm09 positive with Ct values of 21.36 for the matrix gene and 23.41 for the H1pdm09 gene.

In addition, the sample was amplified by one-tube RT-PCR (*1*) and sequenced on the MiSeq platform (Illumina) using the Nextera XT DNA library preparation kit (Illumina) following the manufacturer's instructions.

Consensus sequences for each segment were made using an in-house pipeline. Briefly, raw sequencing reads were quality trimmed using fastp and consensus sequences for each segment were made using an iterative mapping approach with KMA. In a first step, raw reads were mapped against a large reference database with KMA, and the top few reference sequences for each segment were picked for making a reference-based assembly. The reads were then aligned again to this first assembly, and if the two assemblies were identical, this would be outputted as the consensus sequence. If not, the reads would again be aligned to the second assembly and this step would be repeated until the assemblies converged or until a maximum number of iterations.

For phylogenetic analysis, the consensus sequences were aligned to human reference sequences, other swine IAV sequences etc. with MAFFT, alignments were trimmed with trimAl (-gt 0.9 -cons 60) and maximum likelihood phylogenetic trees were built with IQ-TREE using the HKY+G2 method.

Genetic and Antigenic Characterization

Virus isolation was unsuccessful, but antigenic characterization by hemagglutinin inhibition (HAI) test was performed on a culture of the closely related swIAV, A/swine/Denmark/19922–5/2021 (Figure [https://wwwnc.cdc.gov/EID/article/28/12/22-0935-F1.htm]; Appendix 1 Figure 8), which showed poor cross-reactivity to all used reference antisera (Appendix 2, https://wwwnc.cdc.gov/EID/article/28/12/22-0935-App2.xlsx). Also, analysis of the case virus genome sequences showed that it was distinct in all genes (79.7% – 95.3% nucleotide identity) from the contemporary human seasonal influenza vaccine virus A/Victoria/2570/2019 (Table) and contained several differences in the antigenic sites of the Hemagglutinin (HA) protein (Appendix 1 Figure 8). Scanning through protein sequences of all segments, we identified a few amino acid substitutions previously reported to be involved in host specificity, increased pathogenicity or polymerase activity (PB2: T588I; PB1: G216S, Q584H; NP: I100M) (*4*–*7*). The NA protein contained no substitutions known to confer antiviral resistance.

Clinical, Laboratory and Other Examinations Performed to Understand the Severe Course of Illness

A wide range of clinical, laboratory and scanning examinations were performed to reveal any other underlying illness to explain the severe clinical condition of the reported swine flu case.

Microbiological Testing

Microbiological testing included the following patient specimens: Blood culture, cerebrospinal fluid (CSF), tracheal swap, urine sample, fecal sample. The following organisms were tested for using a variety of methods, including culture, microscopy and PCR.

CSF: No observed mononuclear nor neutrophil leukocytes. No observed bacterial nor fungal growth. *Escherichia coli* K1 negative. Haemophilus influenza negative. Listeria monocytogenes negative. Neisseria meningitides negative. Group B streptococcus negative.

Streptococcus pneumonia negative. Epstein-Barr virus negative. Herpes Simplex virus type 1 and 2 negative. Influenza virus A and B negative. Enterovirus negative Cytomegalovirus negative. Human herpesvirus 6 negative. Human parechovirus negative. Varicella zoster virus negative. Cryptococcus neoformans/gattii negative.

Tracheal swap: No fungal growth. **Influenza virus A positive**. Influenza virus B negative. Coronavirus SARS-CoV-2 negative.

Urine: No bacterial or fungal growth.

Blood and CSF Biochemistry

Leukocytes elevated 14.7 × 10E9/L (normal range 3.50–8.80), Erythrocytes normal 0.41 (normal range 0.35–0.46), Hemoglobin 8.3 mmol/L (normal range 7.3–9.5), thrombocytes 216 × 10E9/L (normal range 165–400). Coagulation factors and liver enzymes all within normal ranges.

C-reactive protein (CRP) showed normal levels at time of admission, but was elevated on day 2 at 76 mg/L.

Immunological and inflammatory markers all within normal ranges. Autoimmune encephalitis markers all within normal ranges.

Radiological Examinations

Radiological examinations including chest x-ray, abdominal x-ray, chest CT scan, angiography CT scan, brain CT and MR scan were performed at the time of admission and repeated during hospitalization, which all showed normal conditions and no pathology.

Electroencephalography

To further understand the possible cause of observed convulsions, electroencephalography was performed 2 months after the illness episode, which showed normal conditions.

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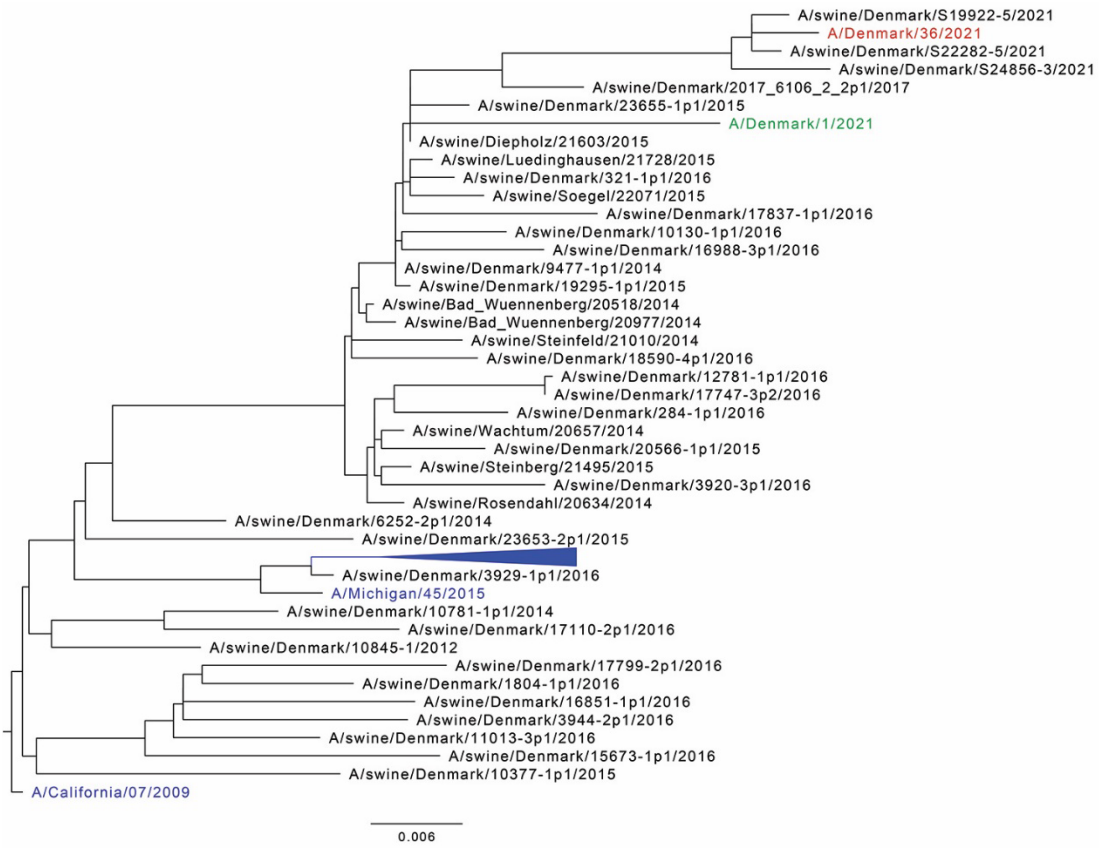
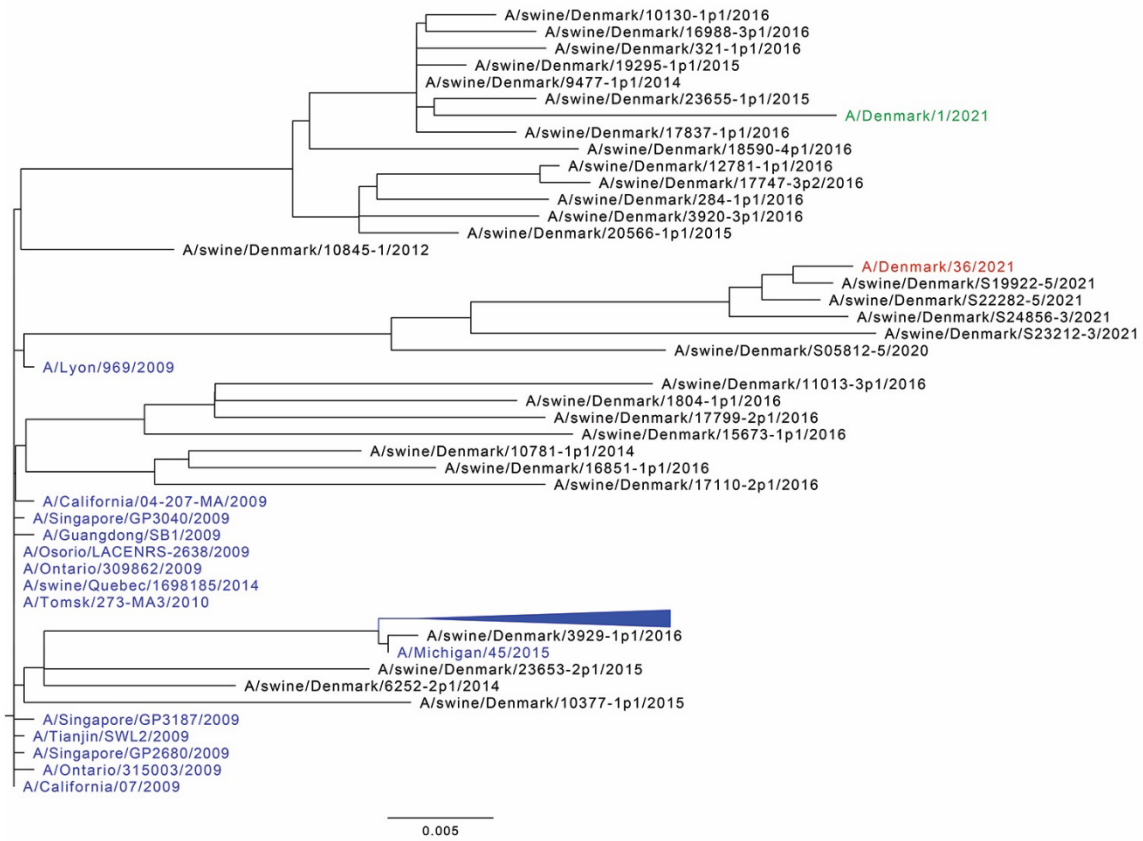
Appendix 1 Table. Results from a Hemagglutination Inhibition test of the closely related swine IAV, A/swine/Denmark/S19922–5/2021 tested against reference ferret antisera of A/H1N1 viruses provided by WHO CC, Francis Crick Institute, UK. Average titer values from duplicate tests of cross-reactivity between the indicated viruses and antisera are indicated. A titer <20 was considered no reaction.

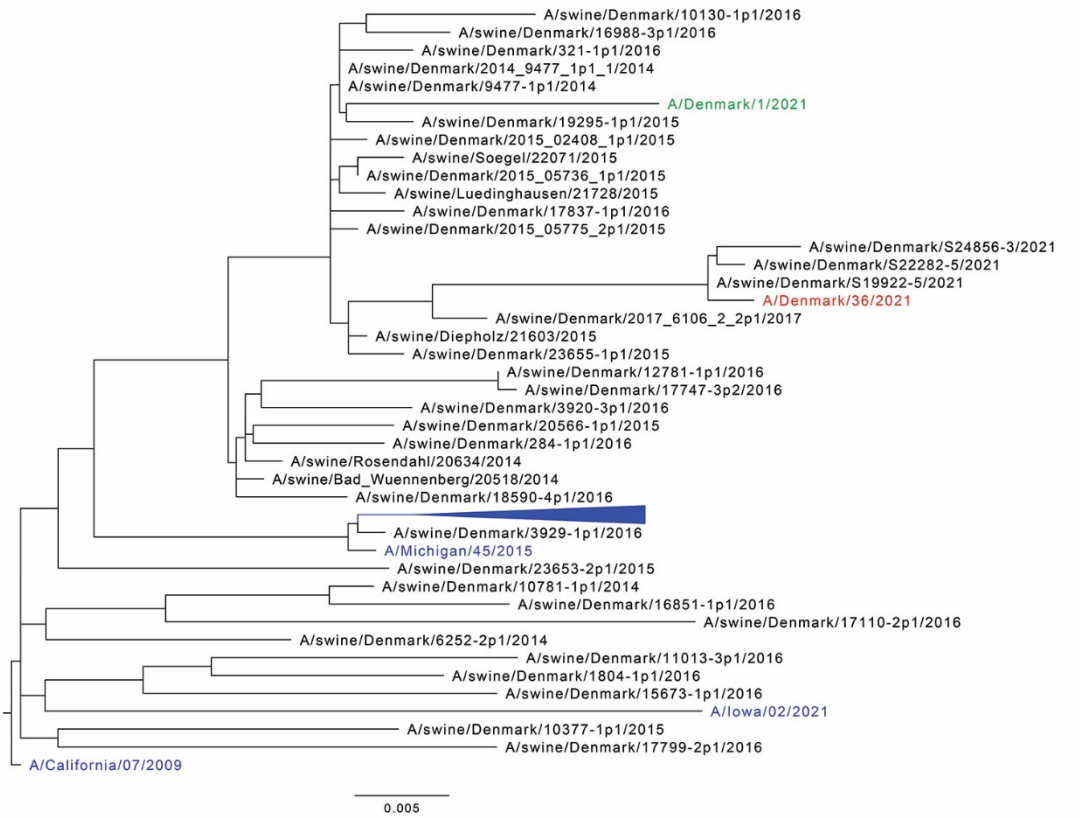
Reference viruses:	A/Victoria/2570/2019	A/Wisconsin/588/2019	A/Denmark/3280/2019	A/Guangdong-Maonan/swl1536/2019	A/California/07/09	A/Michigan/45/2015	A/Brisbane/02/2018
A/Victoria/2570/19	640						
A/Wisconsin/588/2019		1280					
A/Denmark/3280/2019			>2560				
A/Guangdong-Maonan/swl1536/2019				2560			
A/California/07/09					640		
A/Michigan/45/2015						640	
A/Brisbane/02/2018							1280
Sample virus: A/swine/Denmark/S19922–5/2021	<20	<20	<20	<20	<20	<20	<20



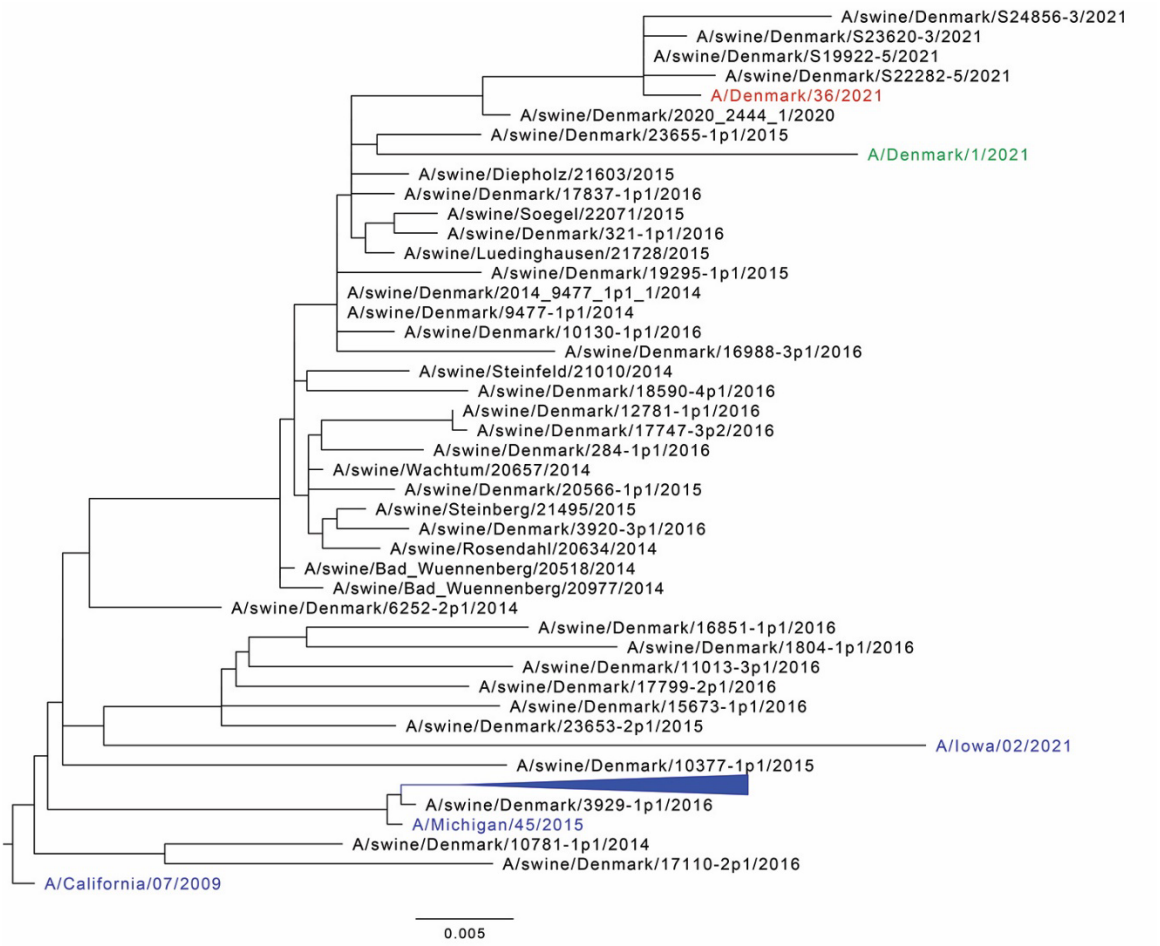
Appendix 1 Figure 1. Maximum-likelihood phylogenetic tree of the Polymerase Basic 2 (PB2) gene segment. The tree includes the case variant virus A/Denmark/36/2021 (red), the 10 closest BLAST matches (https://blast.ncbi.nlm.nih.gov/Blast.cgi), the previously reported Danish variant virus A/Denmark/1/2021 (green), human seasonal reference viruses with >85% nucleotide identity (Appendix 2, https://wwwnc.cdc.gov/EID/article/28/12/22-0935-App2.xlsx), and representative viruses from the passive surveillance program of influenza viruses in Danish pigs. The tree is rooted on A/California/07/2009. Human influenza A virus sequences are shown in blue, and most seasonal reference viruses have been collapsed. The scale bar indicates nucleotide substitutions per site.



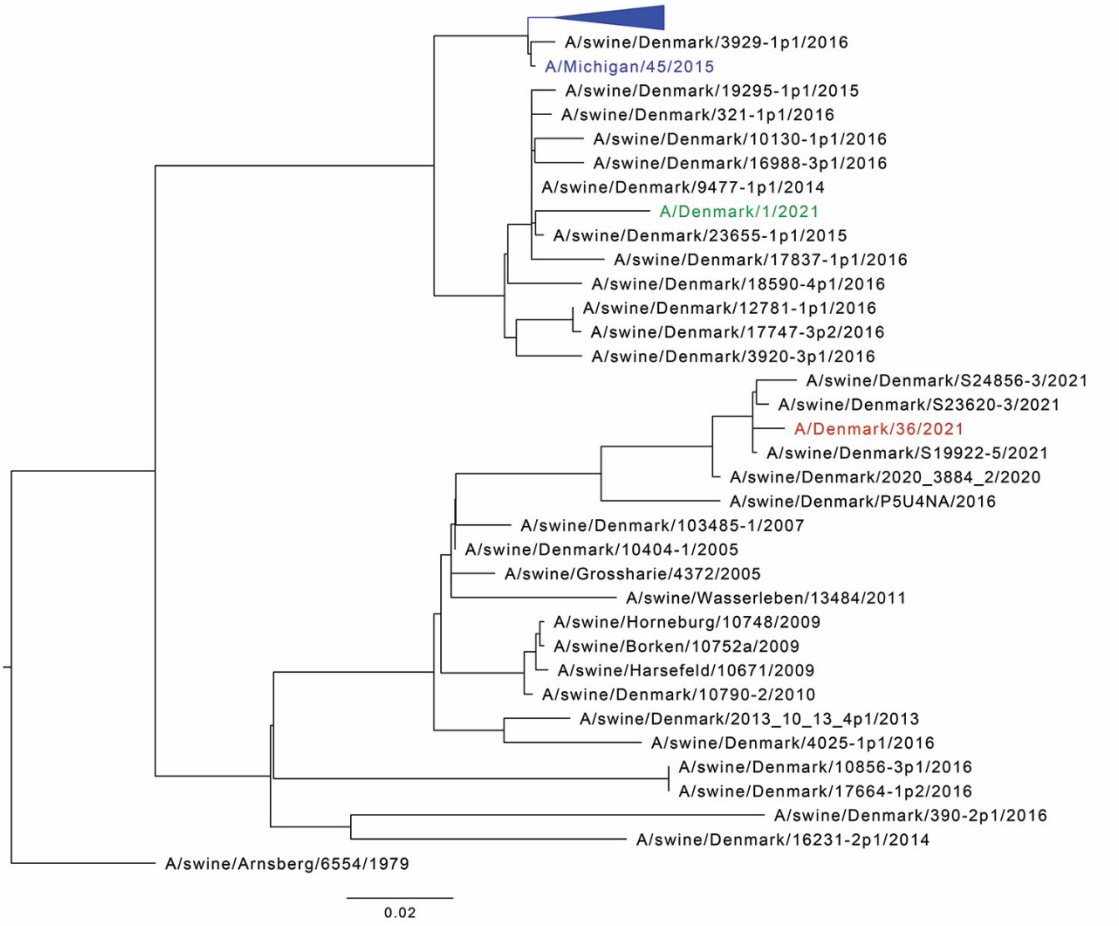
Appendix 1 Figure 2. Maximum-likelihood phylogenetic tree of the Polymerase Basic 1 (PB1) gene segment. The tree includes the case variant virus A/Denmark/36/2021 (red), the 10 closest BLAST matches (https://blast.ncbi.nlm.nih.gov/Blast.cgi), the previously reported Danish variant virus A/Denmark/1/2021 (green), human seasonal reference viruses with >85% nucleotide identity (Appendix 2, https://wwwnc.cdc.gov/EID/article/28/12/22-0935-App2.xlsx), and representative viruses from the passive surveillance program of influenza viruses in Danish pigs. The tree is rooted on A/California/07/2009. Human influenza A virus sequences are shown in blue, and most seasonal reference viruses have been collapsed. The scale bar indicates nucleotide substitutions per site.



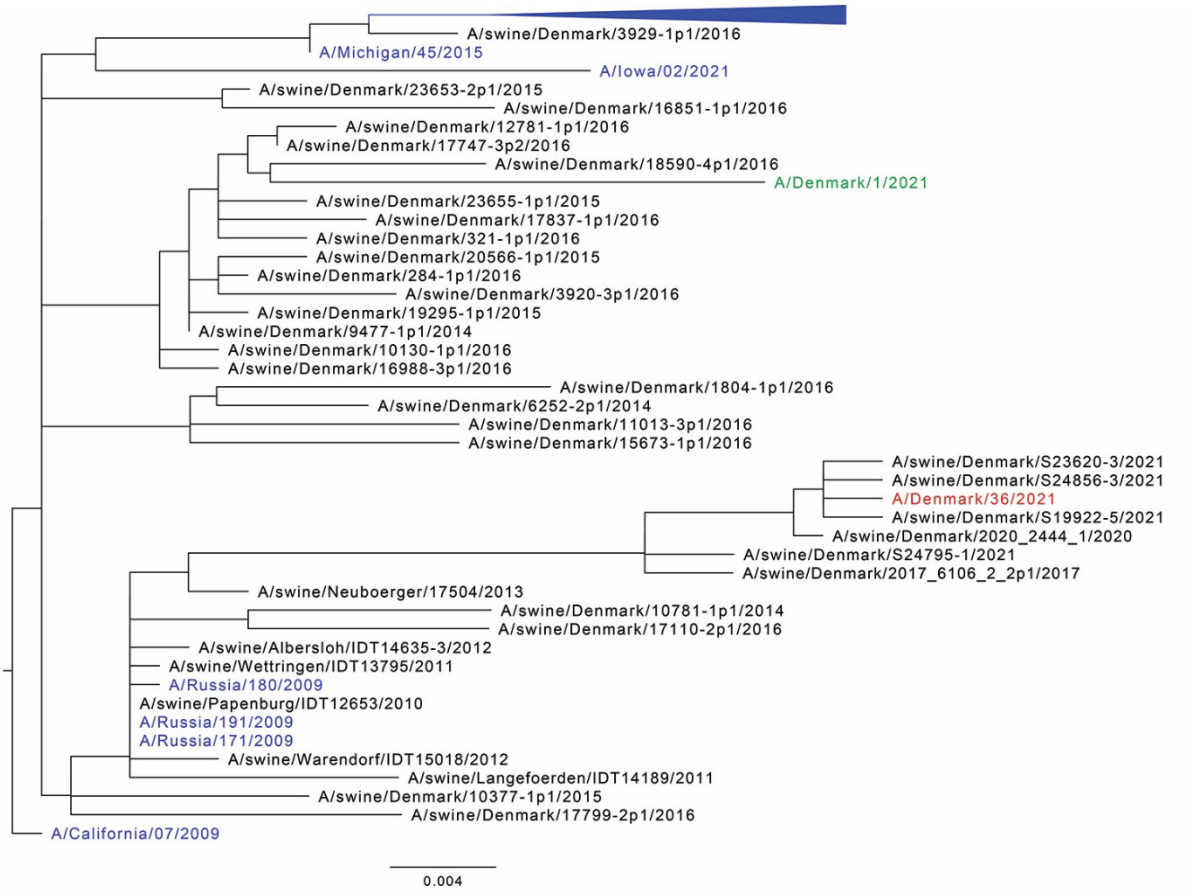
Appendix 1 Figure 3. Maximum-likelihood phylogenetic tree of the Polymerase Acidic (PA) gene segment. The tree includes the case variant virus A/Denmark/36/2021 (red), the 10 closest BLAST matches (https://blast.ncbi.nlm.nih.gov/Blast.cgi), the previously reported Danish variant virus A/Denmark/1/2021 (green), human seasonal reference viruses with >85% nucleotide identity (Appendix 2, https://wwwnc.cdc.gov/EID/article/28/12/22-0935-App2.xlsx), and representative viruses from the passive surveillance program of influenza viruses in Danish pigs. The tree is rooted on A/California/07/2009. Human influenza A virus sequences are shown in blue, and most seasonal reference viruses have been collapsed. The scale bar indicates nucleotide substitutions per site.



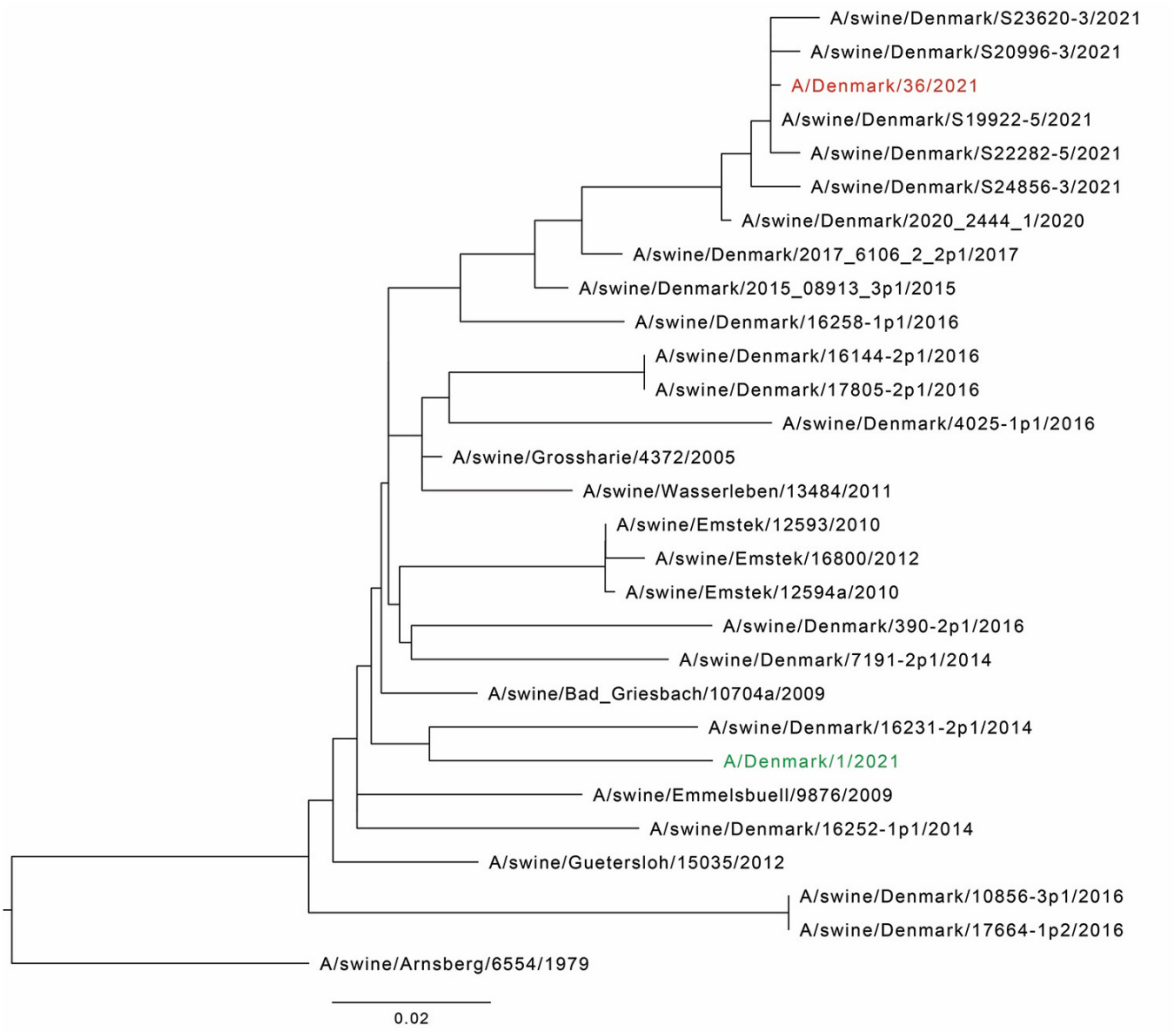
Appendix 1 Figure 4. Maximum-likelihood phylogenetic tree of the Nucleoprotein (NP) gene segment. The tree includes the case variant virus A/Denmark/36/2021 (red), the 10 closest BLAST matches (https://blast.ncbi.nlm.nih.gov/Blast.cgi), the previously reported Danish variant virus A/Denmark/1/2021 (green), human seasonal reference viruses with >85% nucleotide identity (Appendix 2, https://wwwnc.cdc.gov/EID/article/28/12/22-0935-App2.xlsx), and representative viruses from the passive surveillance program of influenza viruses in Danish pigs. The tree is rooted on A/California/07/2009. Human influenza A virus sequences are shown in blue, and most seasonal reference viruses have been collapsed. The scale bar indicates nucleotide substitutions per site.



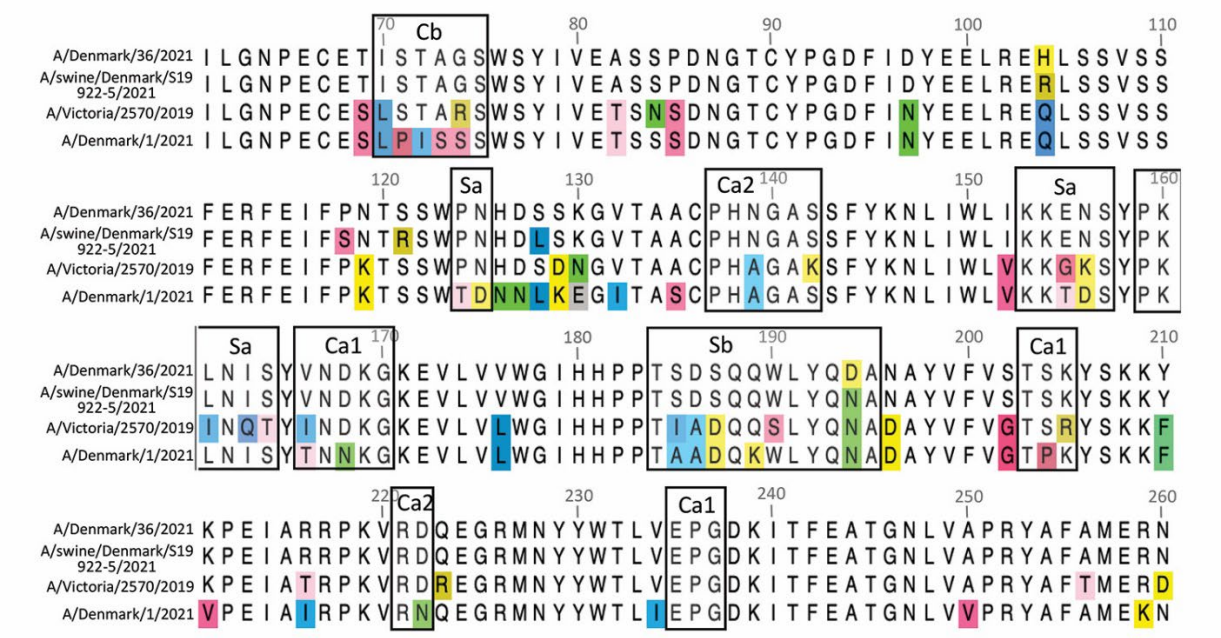
Appendix 1 Figure 5. Maximum likelihood phylogenetic tree of the Neuraminidase (NA) gene segment. The tree includes the case variant virus A/Denmark/36/2021 (red), the ten closest BLAST matches (https://blast.ncbi.nlm.nih.gov/Blast.cgi), the previously reported Danish variant virus A/Denmark/1/2021 (green), human seasonal reference viruses with >85% nucleotide identity (Appendix 2, https://wwwnc.cdc.gov/EID/article/28/12/22-0935-App2.xlsx), and representative viruses from the passive surveillance program of influenza viruses in Danish pigs. The tree is rooted on A/swine/Arnsberg/6554/1979. Human influenza A virus sequences are shown in blue, and most seasonal reference viruses have been collapsed. The scale bar indicates nucleotide substitutions per site.



Appendix 1 Figure 6. Maximum likelihood phylogenetic tree of the Matrix Protein (MP) gene segment. The tree includes the case variant virus A/Denmark/36/2021 (red), the ten closest BLAST matches (https://blast.ncbi.nlm.nih.gov/Blast.cgi), the previously reported Danish variant virus A/Denmark/1/2021 (green), human seasonal reference viruses with >85% nucleotide identity (Appendix 2, https://wwwnc.cdc.gov/EID/article/28/12/22-0935-App2.xlsx), and representative viruses from the passive surveillance program of influenza viruses in Danish pigs. The tree is rooted on A/California/07/2009. Human influenza A virus sequences are shown in blue, and most seasonal reference viruses have been collapsed. The scale bar indicates nucleotide substitutions per site.



Appendix 1 Figure 7. Maximum likelihood phylogenetic tree of the Nonstructural (NS) gene. The tree includes the case variant virus A/Denmark/36/2021 (red), the ten closest BLAST matches (https://blast.ncbi.nlm.nih.gov/Blast.cgi), the previously reported Danish variant virus A/Denmark/1/2021 (green), and representative viruses from the passive surveillance program of influenza viruses in Danish pigs. The tree is rooted on A/swine/Arnsberg/6554/1979. No human seasonal reference viruses had >85% nucleotide identity to this segment. The scale bar indicates nucleotide substitutions per site.



Appendix 1 Figure 8. Alignment of the HA amino acid sequences of the case variant virus A/Denmark/36/2021, the closely related swine influenza virus A/swine/Denmark/S19922–5/2021, the seasonal vaccine strain A/Victoria/2570/2019 (Appendix 2, https://wwwnc.cdc.gov/EID/article/28/12/22-0935-App2.xlsx), and the previously reported Danish variant virus A/Denmark/1/2021. Mutations relative to each other are highlighted in colors, and framed boxes show antigenic sites as defined by Brownlee and Fodor (*2*). Only part of the total HA alignment is shown with H1 numbering starting after the signal peptide (*3*).