Eurasian avian-like swine influenza A(H1N1) viruses (IAVs) are entirely derived from a precursor virus of avian origin (1) and have been enzootic in the swine population in Europe since 1979 and in Asia since 1993. Zoonotic infections with such viruses, which are then termed H1N1 variant (H1N1v) viruses, occur sporadically. Most cases occur in humans who have direct exposure to pigs. Since 1986, several human cases of Eurasian avian-like H1N1 swine IAV have been reported in Europe (2–4) and China (3,5).

These events reflect the possibility of Eurasian avian-like H1N1 swine IAV transmission from swine to humans. In this study, we report an infection with a Eurasian avian-like H1N1 swine IAV in a pig farmer and his pigs in a herd in the Netherlands. We also conducted whole-genome characterization of viruses from the man and the pigs.

The Study
On September 18, 2019, acute respiratory disease was observed in a 43-year-old man (farmer) and his 14-week-old fattening pigs and gilts. The pigs of this closed farm showed coughing, anorexia, tachypnea, dyspnea, and lethargy. Two days earlier, a 44-year-old man (animal caretaker) had reported similar symptoms. The sows of this herd (n = 420) were vaccinated against swine IAVs with Respiporc FLU3 vaccine (Ceva, https://www.ceva.com), but the farmer and animal caretaker were not recently vaccinated against human seasonal influenza viruses. Both humans and the pigs recovered completely within 10 days after the first appearance of signs or symptoms. Family members and close contacts of the men did not show development of influenza-like symptoms.

Six days after onset of disease, nasal swab samples were collected from the farmer, the animal caretaker, and 6 symptomatic pigs. Human samples were collected by self-sampling, and informed consent was obtained from the farmer and the animal caretaker. Subsequently, samples were shipped to the Laboratory of Virology, Faculty of Veterinary Medicine, Ghent University (Merelbeke, Belgium).

Upon inoculation into MDCK cells, IAV was isolated from the sample of the farmer and from a pooled sample of the pigs; no virus was isolated from the animal caretaker. Public health authorities in the Netherlands were notified about the H1N1v infection. The human H1N1v isolate was named A/Netherlands/Gent-193/2019, and the swine H1N1 isolate was named A/swine/Netherlands/Gent-193/2019.

Virus neutralization tests with swine antiserum against swine IAVs of the H1N1, H1N2, and H3N2 subtypes showed an antigenic relationship between the newly discovered isolates and Eurasian avian-like H1N1 swine IAVs from 1998 and 2010, as well as the prototype influenza A(H1N1)pdm09 (pH1N1) A/California/04/2009 virus. Serologic cross-reactivity with H1N2 or H3N2 swine IAVs was not observed (Table 1).

Initial analyses by multiplex real-time reverse transcription PCRs (6) and whole-genome next-generation
sequencing (7) of both isolates confirmed that all genome segments were closely related to those of Eurasian avian-like H1N1 swine IAVs. A BLAST homology search (http://www.fludb.org) with both whole genomes showed highest nucleotide identities (96%) for hemagglutinin (HA) and neuraminidase with clade 1C.2.2 Eurasian avian-like H1N1 swine IAVs isolated in Germany and the Netherlands during 2011–2012. These databases contain limited numbers of sequences of this swine IAV clade, which explains the lack of similar recent viruses. A phylogenetic tree of Eurasian avian-like H1N1 swine IAVs isolated in Europe and Asia was constructed by using MEGA7 software (https://www.megasoftware.net). Phylogenetic analysis confirmed the genetic relationship of the HAI genes of both isolates with Eurasian

Table 1. Cross-reactivity in virus neutralization tests between isolates from a pig farmer and his pigs and reference swine H1N1, pH1N1, H1N2 and H3N2 viruses.

| Virus                  | Subtype | H1 clade | H1 clade | H1 clade | H1 clade | H1 clade | H1 clade | H1 clade | H1 clade | H1 clade |
|------------------------|---------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| swBe98                 | H1N1    | 1C.2     | 256      | 96       | 32       | <4       | <4       | <4       | <4       |<4       |
| swG10                  | H1N1    | 1C.2.1   | 768      | 12       | <4       | <4       | <4       | <4       |<4       |
| Ca09                   | pH1N1   | 1A.3.3.2 | 1,536    | <4       | 768      | <4       | <4       | <4       |<4       |
| swG99                  | H1N2    | 1B.1.2.1 | 6        | <4       | 1,024    |<4       |<4       |
| swG12                  | H1N2    | NA       | <4       |<4       |
| swFl98                 | H3N2    | NA       | <4       |<4       |
| swG08                  | H3N2    | NA       | <4       |<4       |
| Ne19                   | H1N1v   | 1C.2.2   | 128      | 64       |<4       |
| swNe19                 | H1N1v   | 1C.2.2   | 256      | 384      | 1,536    | 8       |<4       |

Table 2. Influenza virus sequences downloaded from GenBank, GISAID, or unpublished data and used in phylogenetic analysis.

Table 2. Influenza virus sequences downloaded from GenBank, GISAID, or unpublished data and used in phylogenetic analysis.

| Isolate                   | Country | Collection date | Date of download | Accession no. |
|---------------------------|---------|-----------------|------------------|---------------|
| A/swine/Finistere/2899/82 | France  | 1982            | 2019 Nov 16      | AJ344015      |
| A/Netherlands/386/86      | Netherlands | 1986            | 2019 Nov 16      | AF320065      |
| A/Netherlands/477/93      | Netherlands | 1993            | 2019 Nov 16      | AF320066      |
| A/swine/Denmark/19126/93  | Denmark  | 1993            | 2019 Nov 16      | KC900289      |
| A/swine/Netherlands/809/96| Netherlands | 1996            | 2019 Nov 16      | AF320064      |
| A/swine/Belgium/1/98      | Belgium  | 1998            | 2019 Nov 17      | AY590624      |
| A/swine/Italy/1513–1/98   | Italy    | 1998            | 2019 Nov 16      | CY116458      |
| A/Switzerland/8808/2002   | Switzerland | 2002            | 2019 Nov 16      | AJ517815      |
| A/swine/Spain/50047/2003  | Spain    | 2003            | 2019 Nov 7       | CY009892      |
| A/swine/Spain/53207/2004  | Spain    | 2004            | 2019 Nov 17      | KR700597      |
| A/swine/Zhejiang/1/2007   | China    | 2007 Nov 15     | 2019 Nov 7       | FJ415610      |
| A/swine/Germany/SV/04/2008| Germany  | 2008 Jun        | 2019 Nov 16      | FN429078      |
| A/swine/Finland/CotesArmor-0388/2009 | France | 2009 Jul 28 | 2019 Nov 17 | KC881265 |
| A/swine/Gent/28/2010      | Belgium  | 2010 Jan 13     | 2019 Jul 29      | KP406525      |
| A/swine/Java/2011         | China    | 2011 Jan 4      | 2019 Nov 23      | KF057112      |
| A/swine/Jiangsu/40/2011   | China    | 2011 Jan 9      | 2019 Nov 23      | JQ319648      |
| A/swine/Germany/Wunnenberg-IDT13220/2011 | Germany | 2011 Mar 31 | 2019 Dec 16 | KR699726 |
| A/swine/Germany/Reinberg-IDT14457–1/2012 | Germany | 2012 Jan 2 | 2019 Dec 16 | KR700366 |
| A/swine/Netherlands/Dalfsen-12/2012 | Netherlands | 2012 Jan 10 | 2019 Dec 16 | KR700020 |
| A/swine/Germany/Ellenbrock-IDT14696/2012 | Germany | 2012 Jan 18 | 2019 Dec 16 | KR700389 |
| A/swine/Gent/62/2015      | Netherlands | 2015 Mar 19 | NA | Unpub. data† |
| A/Hunan/42443/2015       | China    | 2015 Jul 2      | 2020 Jun 15      | EPI206573‡   |
| A/swine/Gent/173/2015     | Belgium  | 2015 Sep 4      | NA               | Unpub.data† |
| A/Pavia/65/2016          | Italy    | 2016 Oct        | 2020 Mar 27      | KY368150      |
| A/Netherlands/3315/2016  | Netherlands | 2016 Oct        | 2020 Mar 27      | KY250319      |
| A/swine/Gent/150/2016    | Belgium  | 2016 Nov 18     | NA               | Unpub.data† |
| A/swine/Gent/196/2018    | Belgium  | 2018 Oct 12     | NA               | Unpub.data† |
| A/swine/Gent/241/2018    | Belgium  | 2018 Nov 4      | NA               | Unpub.data† |
| A/swine/Gent/05/2019     | Belgium  | 2019 Jan 9      | NA               | Unpub.data† |
| A/swine/Gent/54/2019     | Belgium  | 2019 Mar 13     | NA               | Unpub.data† |
| A/swine/Netherlands/Gent-193/2019 | Netherlands | 2019 Sep 24 | 2020 Apr 29 | MT395373 |
| A/Netherlands/Gent-193/2019 | Netherlands | 2019 Sep 24 | 2020 Apr 29 | MT395365 |
| A/swine/Gent/203/2019    | Belgium  | 2019 Oct 9      | NA               | Unpub.data† |

*Accession numbers are from GenBank except as indicated. NA, not applicable.
†Ghent University (Meresbeke, Belgium).
‡GISAID, https://www.gisaid.org.
avian-like H1N1 swine IAVs of clade 1C.2.2 (Table 2; Figure).

The human H1N1v and swine H1N1 isolates differed in several positions in the genes coding for the 3 polymerase proteins (polymerase basic [PB] 2, PB1, and polymerase acidic), the HA gene, and the nonstructural protein gene. The other 3 gene segments (neuraminidase, nucleoprotein, and matrix) were 100% identical. HA gene sequences of the swine H1N1 and the human H1N1v isolates showed amino acid substitutions K142N, N195S, and V215I (H1 numbering). Position 142 is located in antigenic site Ca2 142 and position 195 in antigenic site Sb (8). In human seasonal influenza A(H1N1) viruses, a substitution at position 142 was reported to cause antigenic change (9). This substitution might explain the loss of reactivity with pH1N1 antiserum for the human H1N1v isolate versus the swine H1N1 isolate (Table 1). In addition, in H5 IAVs this substitution decreased the pH at which the HA underwent fusion (10).

Because human-adapted viruses undergo fusion at a lower pH (5.0–5.5) than swine-adapted and avian-adapted viruses (pH 5.6–6.0), such mutations might contribute to human adaptation of zoonotic viruses. We found multiple substitutions in the polymerase genes of the human H1N1v isolate: R739Q in PB2, L108I and T652A in PB1, and D682N in polymerase acidic. Based on analyses in the FluSurver database (http://flusurver.bii.a-star.edu.sg/), the R739Q

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**Figure.** Phylogenetic tree based on amino acid sequences of the hemagglutinin 1 of Eurasian avian-like swine influenza A(H1N1) virus isolates from a pig farmer and his pigs (green circles), the Netherlands, and reference sequences (see Table 2). Red triangles indicate reference sequences from humans. Phylogenetic relationships were estimated by using the maximum-likelihood method in MEGA7 software (https://www.megasoftware.net) and the Jones-Taylor-Thornton substitution model with a gamma distribution of among-site rate. Branch length is proportional to genetic distance. Scale bar indicates amino acid substitutions per site.
substitution in the PB2 gene might influence the binding of PB2 to host protein(s). We also found 2 substitutions in the nonstructural protein gene: V18I and G227R. Sequences were made publicly available in GenBank (accession nos. MT395362–77), and GISAID ([https://www.gisaid.org; accession nos. EPI_ISL_430866 [A/swine/Netherlands/Gent-193/2019] and EPI_ISL_0865 [A/Netherlands/Gent-193/2019]).

Conclusions
We report another zoonotic infection with a Eurasian avian-like H1N1 swine IAV in Europe since the emergence of the virus in 1979 (2–4). No further human-to-human transmission was reported, although it cannot be excluded that the farmer was infected by the animal caretaker. The nasal swab sample from the caretaker might have tested negative because it was collected as late as 8 days after he reported influenza-like symptoms.

The swine antiserum against the pH1N1 virus cross-reacted with the swine H1N1 isolate from this investigation (Table 1) but had a 192-fold lower virus neutralization titer against the human H1N1v isolate. Therefore, it is unlikely that current human seasonal vaccines would provide cross-protection against the human H1N1v isolate. This finding is consistent with our recent investigations of human serum samples for antibodies against 8 H1 swine IAVs representing 7 predominant H1 clades of swine IAVs; only 55 (10%) of 549 human serum samples had hemagglutination inhibition titers ≥40 against a European avian-like H1N1 swine IAV of clade 1C.2.1, which is predominant in swine in Europe (11), compared with 24%–54% against 5 other clades (12). These data point toward a relatively greater zoonotic risk for avian-like H1N1 swine IAVs from Europe and are consistent with previous studies about Eurasian avian-like H1N1 swine IAVs from China (5,13). Our data further support the notion that Eurasian avian-like H1N1 swine IAVs need to be monitored closely.

We found several amino acid substitutions between the H1N1 swine isolate and the H1N1v human isolate, but their role remains obscure. The past 2 decades have seen an unprecedented increase of data for putative mammalian-adaptive mutations of avian influenza viruses. The known genetic markers are mainly based on studies with wholly avian viruses of various HA subtypes in mammalian cell culture or in ferrets. Knowledge of amino acid substitutions that might enable adaptation of swine-adapted influenza viruses to humans, in contrast, is almost nonexistent (14). This finding is true for Eurasian avian-like H1N1 swine IAVs, as well as for the pH1N1 virus, which is the only known swine-origin virus with the ability to spread efficiently between humans. Our study highlights the need for experimental research on this topic and for continued surveillance of swine IAVs because of the risk for human infection or zoonotic spread.

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About the Author
Ms. Parys is a PhD student in the Laboratory of Virology, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium. Her primary research interests are the pig as a model for development of broadly protective influenza A vaccines and public health implications of swine influenza.

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