A zoonotic A/sw/H1, N1 1C.2.2 influenza virus infection was detected in a German child that presented with influenza-like illness, including high fever. There was a history of close contact with pigs 3 days before symptom onset. The child recovered within 3 days. No other transmissions were observed. Serological investigations of the virus isolate revealed cross-reactions with ferret antisera against influenza A(H1N1)pdm09 virus, indicating a closer antigenic relationship with A(H1N1)pdm09 than with the former seasonal H1N1 viruses.

During routine surveillance at the National Influenza Centre in Germany in June 2020, a nasal swab was conspicuous because qPCR for the influenza A virus matrix protein (MP) and N1 neuraminidase (NA) genes were positive, whereas the haemagglutinin (HA) qPCR gave no results. The sample underwent whole genome sequencing and results pointed to a zoonotic influenza virus originating from swine. Here we describe the clinical features of the infection as well as the results of antigenic and genetic characterisation of this zoonotic influenza virus.

Antigenic characterisation

Virus isolation from the child’s nasal swab was successful in MDCK-SIAT cells and embryonated hens’ eggs. The virus was termed influenza A/Hessen/47/2020 (HES/2020). Antigenic characterisation showed that cross-reactivity was highest with swine hyperimmune serum directed against influenza A(H1N1)pdm09 virus, indicating a closer antigenic relationship with A(H1N1)pdm09 than with the former seasonal H1N1 viruses.

Blood samples from 14 of 15 pigs were found to be seropositive against the infecting virus (HES/2020). In haemagglutination inhibition (HI) tests against
HES/2020, titres ranged from 1:10 to 1:160. All pig sera were negative against influenza A(H1N1)pdm09 virus (A/Brisbane/2/2018).

Sequence analysis showed that the majority of HA antigenic sites were conserved between influenza A/sw/H1avN1 and A(H1N1)pdm09 viruses (Table 2) [2]. In accordance with International Health Regulations, the case was reported to World Health Organization (WHO) via the Early Warning and Response System (EWRS) [3] and the virus was provided to the WHO Collaborating Centre London for further characterisation [4].

Genetic characterisation

The genetic classification of HES/2020 is F (polymerase basic protein 2, PB2), G (polymerase basic protein 1, PB1), I (polymerase acidic protein, PA), 1C.2.2 (HA), F (nucleoprotein, NP), 1F (NA), F (MP), 1E (nonstructural proteins, NS) [5,6]. It is unrelated to the recently reported G4 reassortant EA(H1N1) viruses circulating in China [2]. Sequences were submitted to GISAID and the accession numbers were as follows: PB2: EPI1757436, PB1: EPI1757437, PA: EPI1757435, HA: EPI1757439, NP: EPI1757432, NA: EPI1757438, MP: EPI1757434 and NS: EPI1757433. Blast analysis and phylogenetic analysis demonstrated that the segments of HES/2020 are closely related to those of different viruses: HA (Figure) and NA to influenza A/swine/Germany/Ellerbrock-IDT14696/2012 (swELLE/2012, H1N1, HA-1C.2.2) and A/swine/Duelmen/15075/2012 (swDUEL/2012, H1N1, HA-1C.2.2); MP, NP, NS and PB1 to A/swine/Luedinghausen/18391/2013 (swLUED/2013, H1N1, HA-1C.2.1) and to zoonotic A/Netherlands/3315/2016 (NL/2016, H1N1, HA-1C.2.1) [7]; PA and PA-X to A/swine/Belgium/Heist-op-den-Berg-363/2012 (swHEIST/2012, H1N1, HA-1C.2.1); and PB2 to A/swine/Belgium/Oostkamp-26/2012 (swOOST/2012, H1N2, HA-1B.1.2.1). The genetic composition of HES/2020 indicates several intra- and inter-clade reassortments.

Nucleotide sequence variation was highest over the usually well conserved NP and PA-X coding sequences (Twelve coding sequences were analysed: HA, NA, M1, M2, NP, NS1, NEP, PA, PA-X, PB1, PB1-F2, PB2 with a length of 1,701, 1,410, 759, 294, 1,497, 693, 366, 2,151, 759, 2,274, 273, 2,280 nt, respectively). They displayed nucleotide identities of 95% each, whereas all other coding sequences displayed nucleotide sequence identity 95% relative to the reference sequence. Reference sequences were swDUEL/2012 and swOOST/2012.
for HA and NA, swLUED/2013 for MP, NP, NS and PB1, swHEIST/2012 for PA and swOOST/2012 for PB2). Amino acid (AA) sequence variation was highest over the regulator proteins of the host innate immune response, NS1, PA-X and PB1-F2 (identities of 95%, 94% and 95%, respectively) [8,9]. Variant calling for HES/2020 and another zoonotic virus, NL/2016 [7], relative to the reference viruses, demonstrated that the number of substitutions common to both HES/2020 and another zoonotic virus, NL/2016, was highest for the PB1-F2 protein (four of five substitutions) Table 3). In contrast to NL/2016, PB1-F2 of HES/2020 is full-length at 90 AA. Phylogenetic analyses of MP, NP, NS, NS1, PB1 and PB1-F2 demonstrated that the two zoonotic viruses are closely related (Figure, Supplementary Figures S1–S10). To detect substitutions with potential functional relevance in the HES/2020 genome, the FluSurver online tool was employed (https://flusurver.bii.a-star.edu.sg/), identifying substitutions in the HA receptor binding domain (D222E) [10], NP (K48Q;R98K;R99K [11], R351K;V353I;Q357K [12]) and PB2 (D701N) [13] (Supplementary Table S1). The substitutions NP-Q357K, PA-X-R57K, PA-R57K, PA-T639A are present in both zoonotic viruses and in both analyses (FluSurver and the genetic comparison in Table 3).

**Resistance characterisation**

While HES/2020 does not exhibit NA or PA mutations conferring resistance against neuraminidase inhibitors or baloxavir marboxil, its M2 sequence contains the AA substitutions L26I, V27A and S31N, all of which are associated with adamantane resistance (amantadine and rimantadine). Phenotypic susceptibility testing against oseltamivir, peramivir and zanamivir confirmed that HES/2020 was sensitive to all neuraminidase inhibitors authorised in Europe.

**Discussion**

This is the sixth zoonotic swine influenza virus infection in humans investigated at the German National Influenza Centre (in 2007: A/swine/H1N1 and A/swine/H3N2 in Lower Saxony, in 2010: A/swine/H1N1 in Lower Saxony, in 2011: A/swine/H1N2 and A/swine/H1N1 in Lower Saxony) [14]. Of the five previously reported cases, two occurred in children and one in an immuno-compromised adult; influenza A/sw/H1N1 infections.

**Table 2**

Comparison of amino acids in the antigenic sites of the haemagglutinin molecule of HES/2020 vs influenza A(H1N1) viruses, Germany, June 2020

| Site Sa | Amino acid in the antigenic site* |
|----------------|----------------------------------|
| Virus HA clade/genotype | 124 | 125 | 155 | 157 | 159 | 160 | 162 | 163 | 164 | 153 | 156 | 185 | 189 | 190 | 193 | 195 |
| HES/2020 1C.2.2 | P | N | G | S | P | K | R | N | S | K | N | D | Q | T | Q | N |
| swDUEL/2012 1C.2.2 | P | N | G | S | P | K | R | K | S | K | N | D | Q | T | Q | N |
| swLUED/2013 1C.2.1 | P | N | G | S | P | K | S | T | S | K | N | D | Q | T | Q | N |
| NL/2016 1C.2.1 | P | N | E | S | P | K | S | T | S | K | N | D | Q | T | Q | N |
| swSHA/2013 1C.2.3/61 | P | N | G | S | P | K | S | T | S | K | N | D | Q | T | Q | N |
| swHEN/2018 1C.2.3/G4 | P | N | G | S | P | K | S | S | S | K | N | D | Q | T | Q | N |
| swSHA/2014 1C.2.3/G5 | P | N | G | S | P | K | S | S | S | K | N | D | Q | T | Q | N |
| swANH/2015 1C.2.3/G6 | P | N | G | S | P | K | S | S | S | K | N | D | Q | T | Q | N |
| GU-MA/2019 pdm09 | P | N | G | S | P | K | N | Q | T | K | N | I | E | S | Q | A |
| MIC/2015 pdm09 | P | N | G | S | P | K | N | Q | S | K | N | T | Q | S | Q | A |
| Site Sb | Amino acid in the antigenic site* |
|----------------|----------------------------------|
| Virus HA clade/genotype | 166 | 170 | 204 | 237 | 135 | 137 | 142 | 221 | 222 | 70 | 71 | 73 | 74 | 75 | 115 |
| HES/2020 1C.2.2 | T | G | S | G | A | S | G | N | R | E | L | L | A | N | S | E |
| swDUEL/2012 1C.2.2 | T | G | S | G | A | S | G | N | R | E | L | L | A | N | S | E |
| swLUED/2013 1C.2.1 | T | G | S | G | A | S | G | K | R | E | L | I | A | N | S | E |
| NL/2016 1C.2.1 | T | G | S | G | A | S | G | K | R | E | L | I | A | N | S | E |
| swSHA/2013 1C.2.3/61 | T | G | S | G | A | S | G | N | R | G | L | L | A | N | S | E |
| swHEN/2018 1C.2.3/G4 | T | G | T | G | S | S | G | N | R | E | L | L | A | N | S | E |
| swSHA/2014 1C.2.3/G5 | T | G | S | G | S | S | G | N | R | E | L | L | A | N | S | E |
| swANH/2015 1C.2.3/G6 | T | G | S | G | A | S | G | N | R | E | L | L | A | N | S | E |
| GU-MA/2019 pdm09 | I | G | S | G | A | P | G | K | R | D | L | S | A | R | S | E |
| MIC/2015 pdm09 | I | G | S | G | A | P | G | K | R | D | L | S | A | S | S | E |

HA: haemagglutinin.
* H1 numbering without signal sequence.

Virus names from top to bottom: A/Hessen/47/2020, A/swine/Duelmen/35075/2012, A/swine/Luedinghausen/18391/2013, A/Netherlands/3315/2016, A/swine/Shandong/39/2013, A/swine/Henan/SN13/2018, A/swine/Shandong/S113/2014, A/swine/Anhui/1227/2015, A/Guangdong-Maonan/SWL1536/2019, A/Michigan/45/2015.

Shaded cells: amino acid differences relative to HES/2020; presentation of antigenic sites adapted from [2].

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Phylogenetic analysis of the haemagglutinin gene (1,695 bp) of influenza A viruses

The phylogenetic analyses of the other coding sequences (NA, MP, NP, NS, NS1, PA, PA-X, PB1, PB1-F2, PB2) are shown in Supplementary Figures S1–S10. Virus genomes were analysed by whole genome sequencing and were phylogenetically evaluated with Mega7 (neighbour-joining method, midpoint rooted, bootstrap test with 1,000 replicates, Kimura 2-parameter method, partial deletion (site coverage cut-off: 5%). Sixty-one influenza A viruses were characterised: 1A.3.3.2/H1N1pdm09 (light blue), 1B.1.2.1 (black), 1C.1 (grey), 1C.2 including reassorted A(H1N2)-viruses (orange), 1C.2.1 (green), 1C.2.2 including zoonotic A/Hessen/47/2020 (red, italics, framed in black) and 1C.2.3 including genotypes G1/G4/G5/G6 (blue) [2]. Framed items: closely related viruses that are identified by BLAST analysis of each segment (data not shown) and used as reference viruses for further analysis: swELLE/2012 and swDUEL/2012 for HA and NA, zoonotic NL/2016 and swLUED/2013 for MP, NP, NS and PB1, swHEIST/2012 for PA and swOOST/2012 for PB2.
were the most common [14]. All previous German cases were detected in Lower Saxony, the federal state with the second largest pig population in Germany. The case described here is the first from a region with a low density of pig holdings, i.e. Hesse.

The genetic diversity of influenza A viruses in the European pig population is increasing [15-17]. A/sw/H1_N1 are the predominant swine influenza viruses in Germany [18]. Among them, the two most prevalent lineages are H1_N1 1C.2.2 and H1_N1 1C.2.1. Other swine influenza viruses include H1_N2 and H3_N2 viruses as well as H1N1 and H1pdm_N2 viruses [15-18]. An increasing number of reassortments between these viruses augment the diversity of influenza virus populations in swine.

Swine influenza viruses acquired adamantane resistance in the late 1980s [19]. The influenza A(H1N1)pdm09 virus contains the MP gene from A/sw/H1avN1 virus and A/sw/H1avN1 viruses in ferrets. This is in line with previous findings that influenza A(H1N1)pdm09 infection induces broadly neutralising (not strain-specific) antibodies [26]. Antibodies against influenza A/sw/H1_N1 viruses in the human population are rare [27,28]. On the other hand, sera of human volunteers collected 3–7 weeks after vaccination with the annual 2017/18 vaccine all reflected antibodies against influenza A/sw/H1avN1 virus at varying microneutralisation titres and none was negative [15]. Although the family members of the zoonotic case had not been vaccinated, they may have been exposed to human and swine influenza A viruses before, potentially resulting in pre-existing immunity which might impair transmission of influenza A/sw/H1avN1 influenza virus.

However, the rising genetic diversity among swine influenza viruses, involving antigenic drift and shift, may increase divergence from influenza A/sw/H1avN1 viruses in the future. In particular, swine reassortant influenza viruses may quickly acquire antigenic changes, and this is where substantial zoonotic potential may arise.

Our serology investigations indicate some level of cross-reactivity between influenza A(H1N1)pdm09 virus and A/sw/H1avN1 viruses in ferrets. This is in line with previous findings that influenza A(H1N1)pdm09 infection induces broadly neutralising (not strain-specific) antibodies [26]. Antibodies against influenza A/sw/H1avN1 viruses in the human population are rare [27,28]. On the other hand, sera of human volunteers collected 3–7 weeks after vaccination with the annual 2017/18 vaccine all reflected antibodies against influenza A/sw/H1avN1 virus at varying microneutralisation titres and none was negative [15]. Although the family members of the zoonotic case had not been vaccinated, they may have been exposed to human and swine influenza A viruses before, potentially resulting in pre-existing immunity which might impair transmission of influenza A/sw/H1avN1 influenza virus.

Swine influenza viruses have acquired some resistance genes against human myxovirus resistance protein MxA during their evolution in pigs, facilitating their transmission to humans [12]. Pig-to-human influenza virus transmissions are not rare, especially in close contact settings such as agricultural fairs [22], and sporadic zoonotic transmission of swine influenza A(H1N1) virus has been reported [23,24]. The farm child was the only member of his family who was infected, although some of the other family members had also been exposed. The infection of a child is not surprising. Because of their limited exposure history, young children display a narrower (if any) immune response to influenza virus than adults [25].
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Conflict of interest

None declared.

Authors’ contributions

RD and WH designed the study, RD, MW, DYO, SD wrote the manuscript, RD, MW, BB, MH-K, CG, RV, AMH, KG, AT, SA, JR, SD, SB, TW contributed to the investigations, all authors read and edited the manuscript.

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