

Supplementary information for

Zoonotic infection with swine A/H1_{av}N1 influenza virus in a German child

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Table S1

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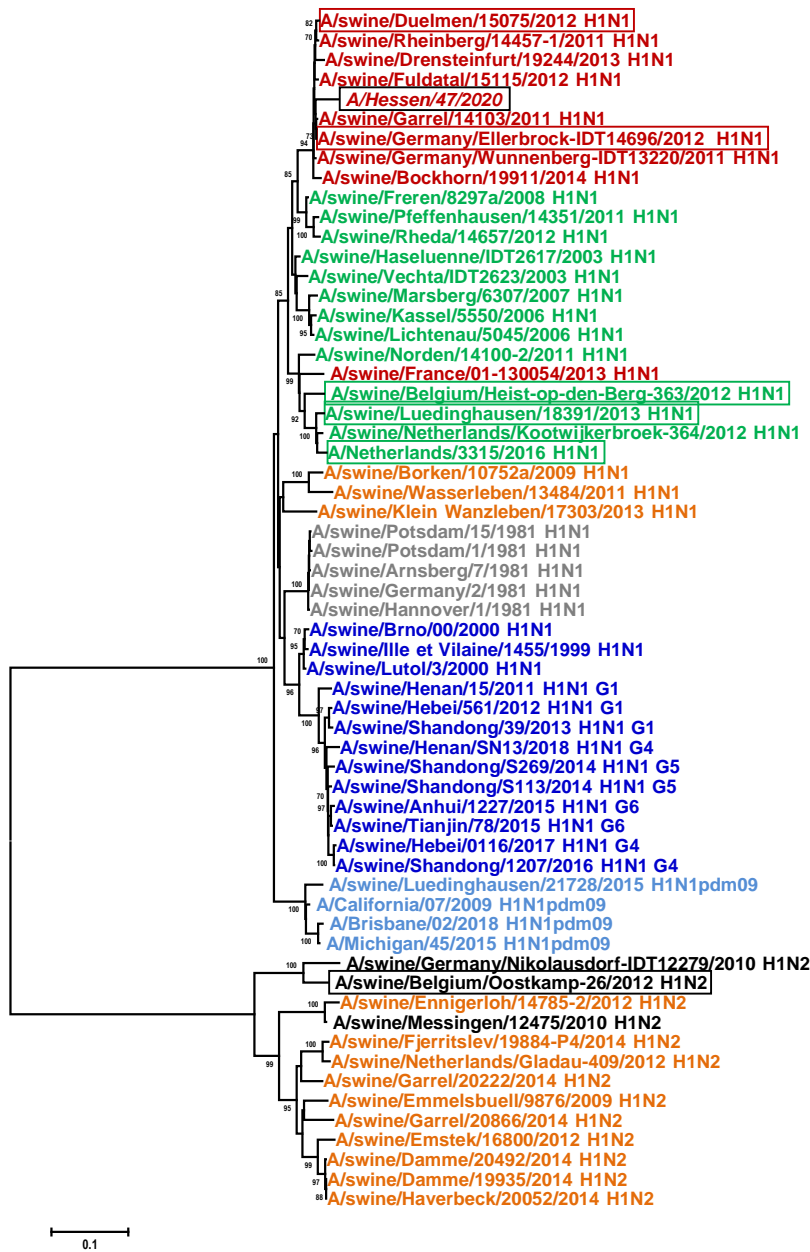


Figure S1: Phylogenetic analysis of NA gene of influenza A viruses. Virus genomes were analyzed by NGS and were phylogenetically evaluated with Mega7 (Neighbor-Joining method, midpoint rooted, bootstrap test with 1000 replicates, Kimura 2-parameter method, partial deletion (site coverage cutoff 5%). Sixty-one influenza A viruses were characterized: 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, Sun 2020 [3]). Closely related viruses that are identified by Blast analysis of each segment (data not shown) and used as reference viruses for further analysis, respectively, are framed: For HA and NA, swELLE/2012 and swDUEL/2012; for MP, NP, NS, NS1, PB1 and PB1-F2, zoonotic NL/2016 and swLUED/2013; for PA and PA-X, swHEIST/2012; and for PB2, swOOST/2012

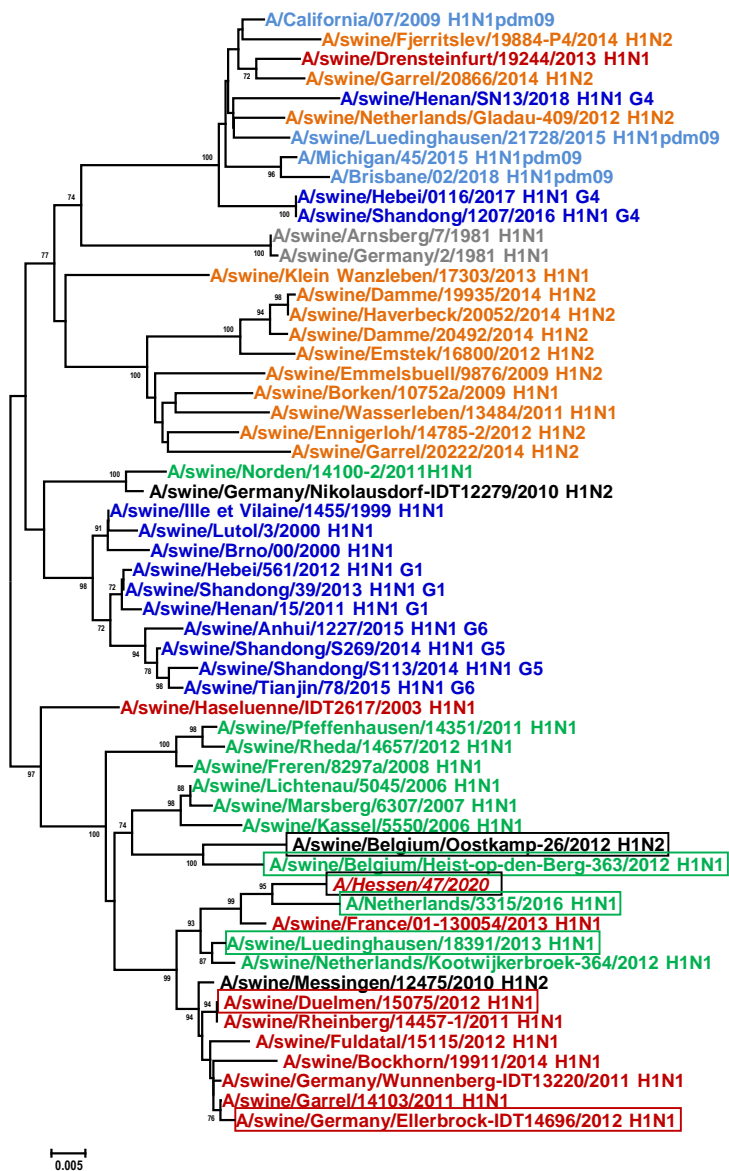


Figure S2: Phylogenetic analysis of MP gene of influenza A viruses. Virus genomes were analyzed by NGS and were phylogenetically evaluated with Mega7 (Neighbor-Joining method, midpoint rooted, bootstrap test with 1000 replicates, Kimura 2-parameter method, partial deletion (site coverage cutoff 5%). Sixty-one influenza A viruses were characterized: 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, Sun 2020 [3]). Closely related viruses that are identified by Blast analysis of each segment (data not shown) and used as reference viruses for further analysis, respectively, are framed: For HA and NA, swELLE/2012 and swDUEL/2012; for MP, NP, NS, NS1, PB1 and PB1-F2, zoonotic NL/2016 and swLUED/2013; for PA and PA-X, swHEIST/2012; and for PB2, swOOST/2012

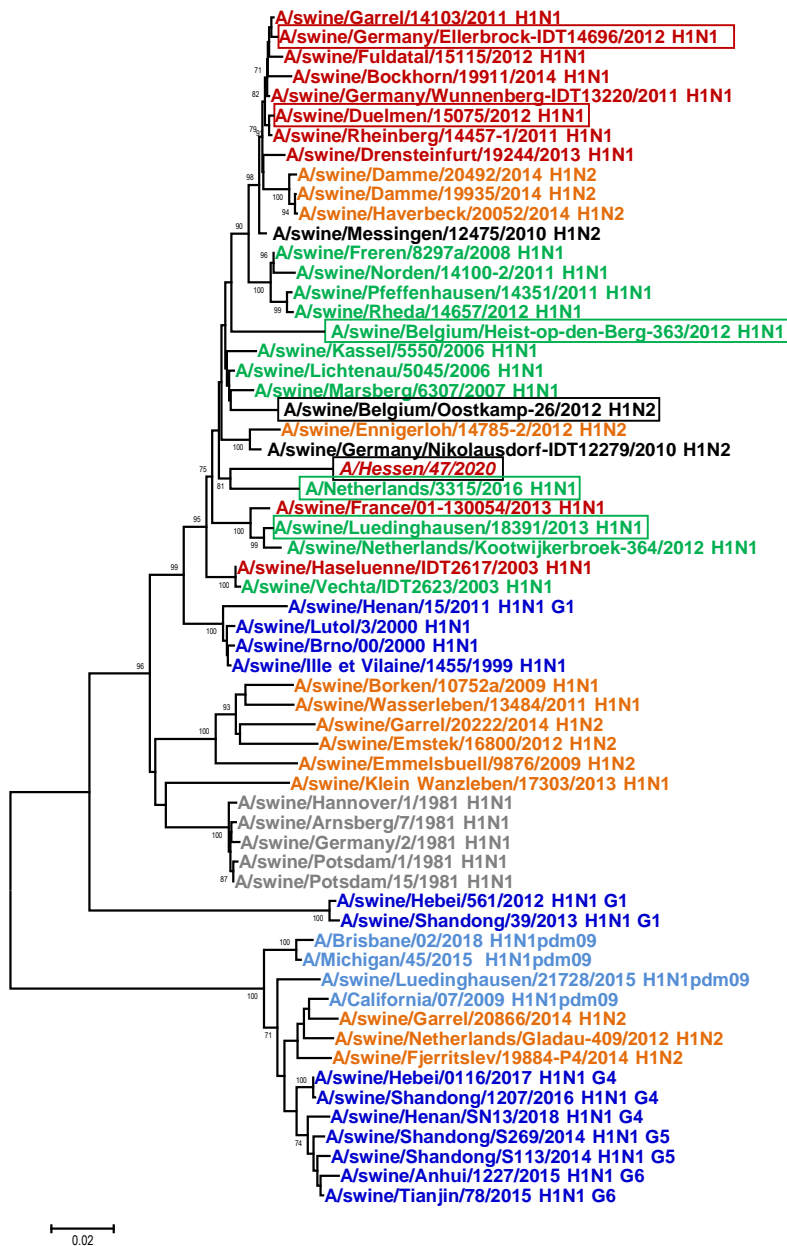


Figure S3: Phylogenetic analysis of NP gene of influenza A viruses. Virus genomes were analyzed by NGS and were phylogenetically evaluated with Mega7 (Neighbor-Joining method, midpoint rooted, bootstrap test with 1000 replicates, Kimura 2-parameter method, partial deletion (site coverage cutoff 5%). Sixty-one influenza A viruses were characterized: 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, Sun 2020 [3]). Closely related viruses that are identified by Blast analysis of each segment (data not shown) and used as reference viruses for further analysis, respectively, are framed: For HA and NA, swELLE/2012 and swDUEL/2012; for MP, NP, NS, NS-1, PB1 and PB1-F2, zoonotic NL/2016 and swLUED/2013; for PA and PA-X, swHEIST/2012; and for PB2, swOOST/2012

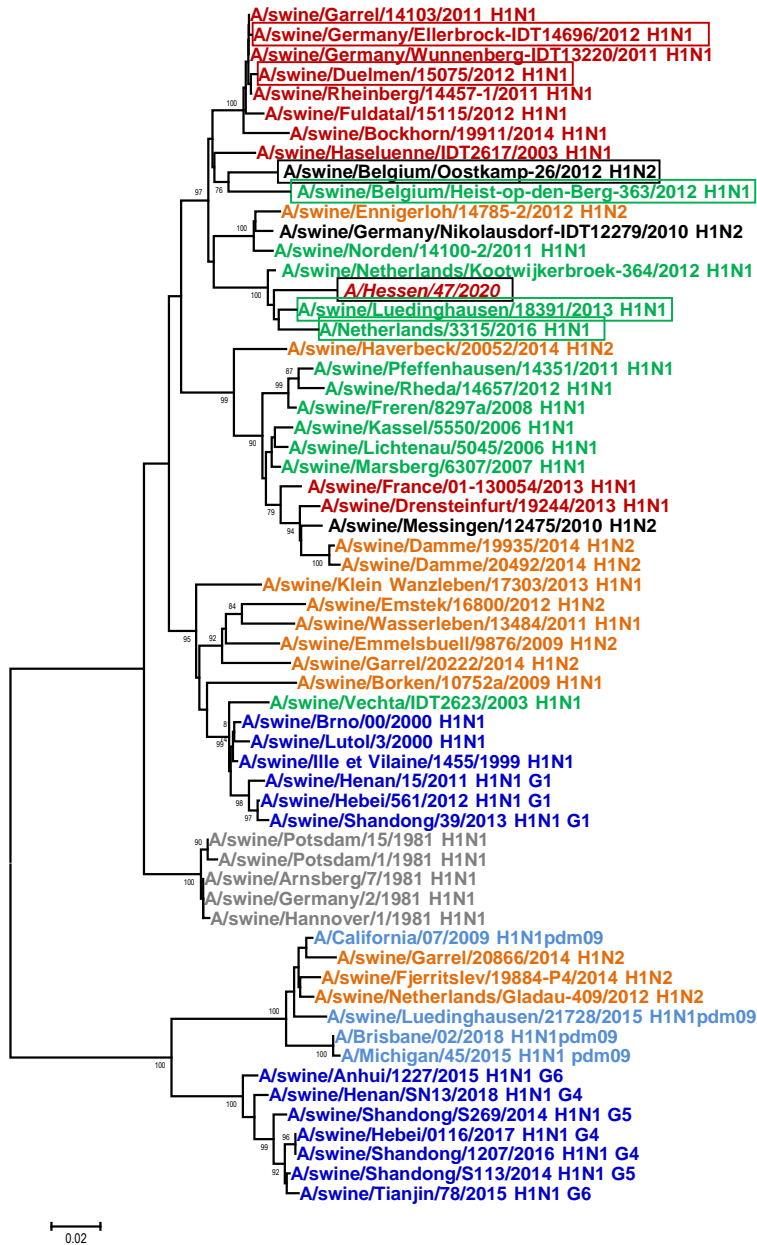


Figure S4: Phylogenetic analysis of NS gene of influenza A viruses. Virus genomes were analyzed by NGS and were phylogenetically evaluated with Mega7 (Neighbor-Joining method, midpoint rooted, bootstrap test with 1000 replicates, Kimura 2-parameter method, partial deletion (site coverage cutoff 5%). Sixty-one influenza A viruses were characterized: 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, Sun 2020 [3]). Closely related viruses that are identified by Blast analysis of each segment (data not shown) and used as reference viruses for further analysis, respectively, are framed: For HA, NA, swELLE/2012 + swDUEL/2012; for MP, NP, NS, NS1, PB1, PB1-F2, zoonotic NL/2016 and swLUED/2013; for PA, PA-X, swHEIST/2012; and for PB2, swOOST/2012

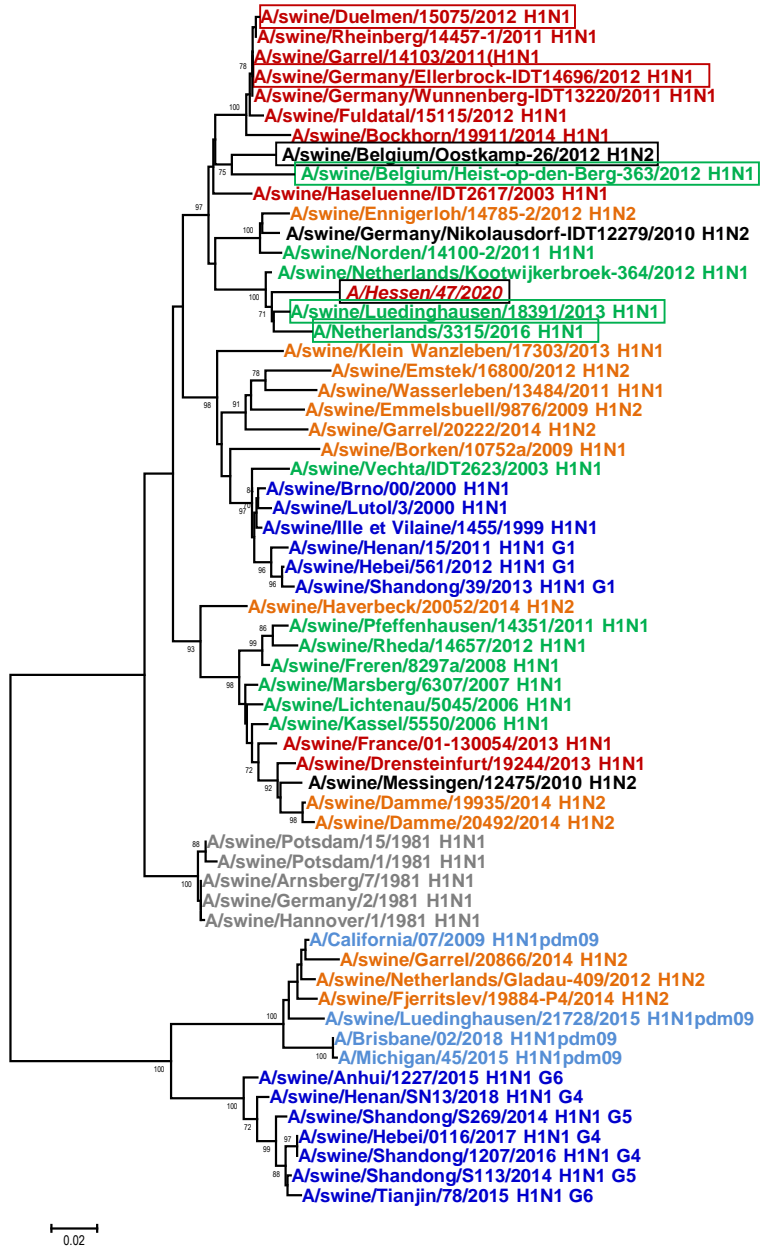


Figure S5: Phylogenetic analysis of NS1 gene of influenza A viruses. Virus genomes were analyzed by NGS and were phylogenetically evaluated with Mega7 (Neighbor-Joining method, midpoint rooted, bootstrap test with 1000 replicates, Kimura 2-parameter method, partial deletion (site coverage cutoff 5%). Sixty-one influenza A viruses were characterized: 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, Sun 2020 [3]). Closely related viruses that are identified by Blast analysis of each segment (data not shown) and used as reference viruses for further analysis, respectively, are framed: For HA, NA, swELLE/2012 and swDUEL/2012; for MP, NP, NS, NS1, PB1, PB1-F2, zoonotic NL/2016 + swLUED/2013; for PA, PA-X, swHEIST/2012; and for PB2, swOOST/2012

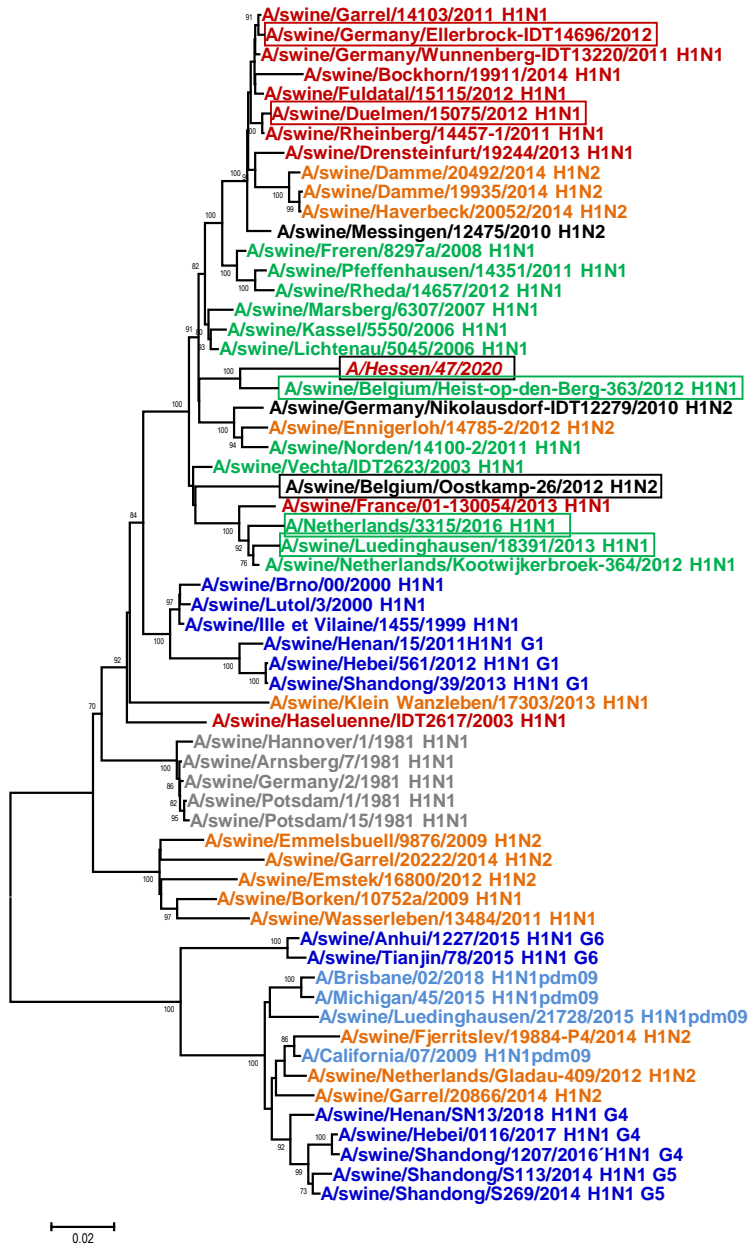


Figure S6: Phylogenetic analysis of PA gene of influenza A viruses. Virus genomes were analyzed by NGS and were phylogenetically evaluated with Mega7 (Neighbor-Joining method, midpoint rooted, bootstrap test with 1000 replicates, Kimura 2-parameter method, partial deletion (site coverage cutoff 5%). Sixty-one influenza A viruses were characterized: 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, Sun 2020 [3]). Closely related viruses that are identified by Blast analysis of each segment (data not shown) and used as reference viruses for further analysis, respectively, are framed: For HA, NA, swELLE/2012 and swDUEL/2012; for MP, NP, NS, NS1, PB1, PB1-F2, zoonotic NL/2016 + swLUED/2013; for PA, PA-X, swHEIST/2012; and for PB2, swOOST/2012

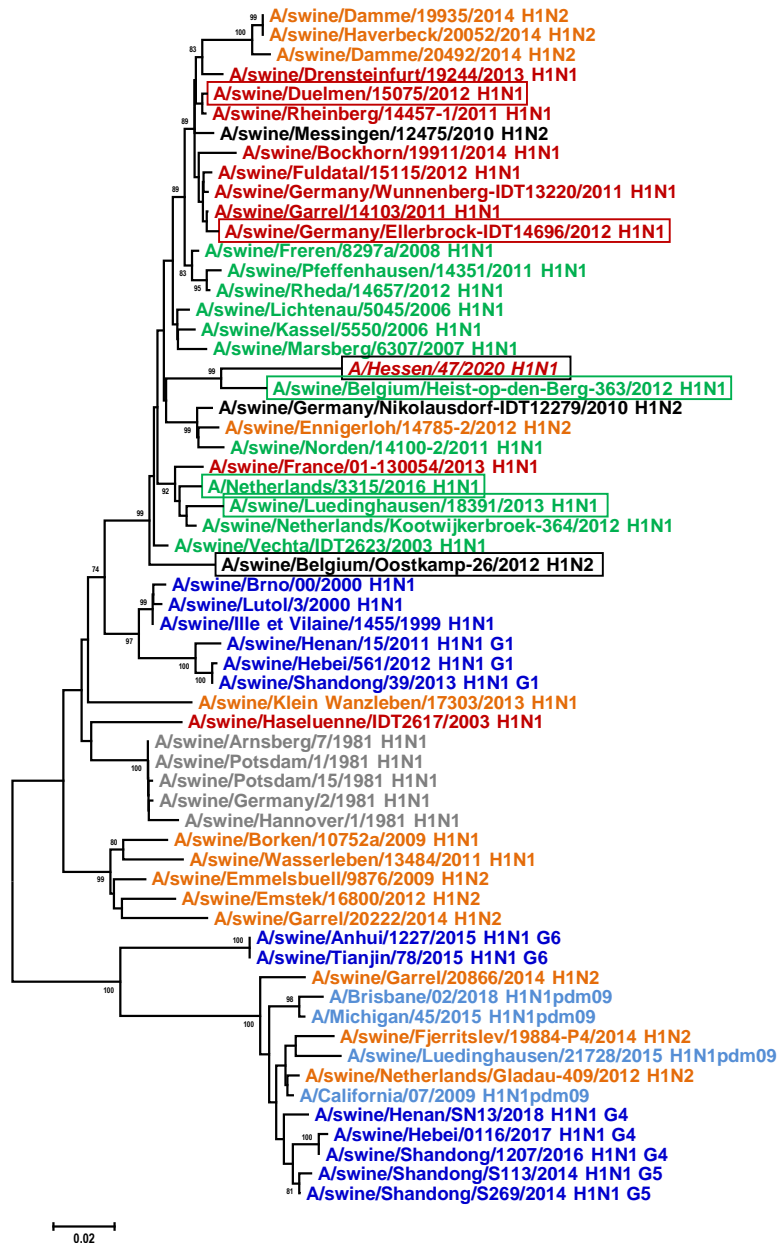


Figure S7: Phylogenetic analysis of PA-X gene of influenza A viruses. Virus genomes were analyzed by NGS and were phylogenetically evaluated with Mega7 (Neighbor-Joining method, midpoint rooted, bootstrap test with 1000 replicates, Kimura 2-parameter method, partial deletion (site coverage cutoff 5%). Sixty-one influenza A viruses were characterized: 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, Sun 2020 [3]). Closely related viruses that are identified by Blast analysis of each segment (data not shown) and used as reference viruses for further analysis, respectively, are framed: For HA, NA, swELLE/2012 and swDUEL/2012; for MP, NP, NS, NS1, PB1, PB1-F2, zoonotic NL/2016 and swLUED/2013; for PA, PA-X swHEIST/2012; and for PB2, swOOST/2012

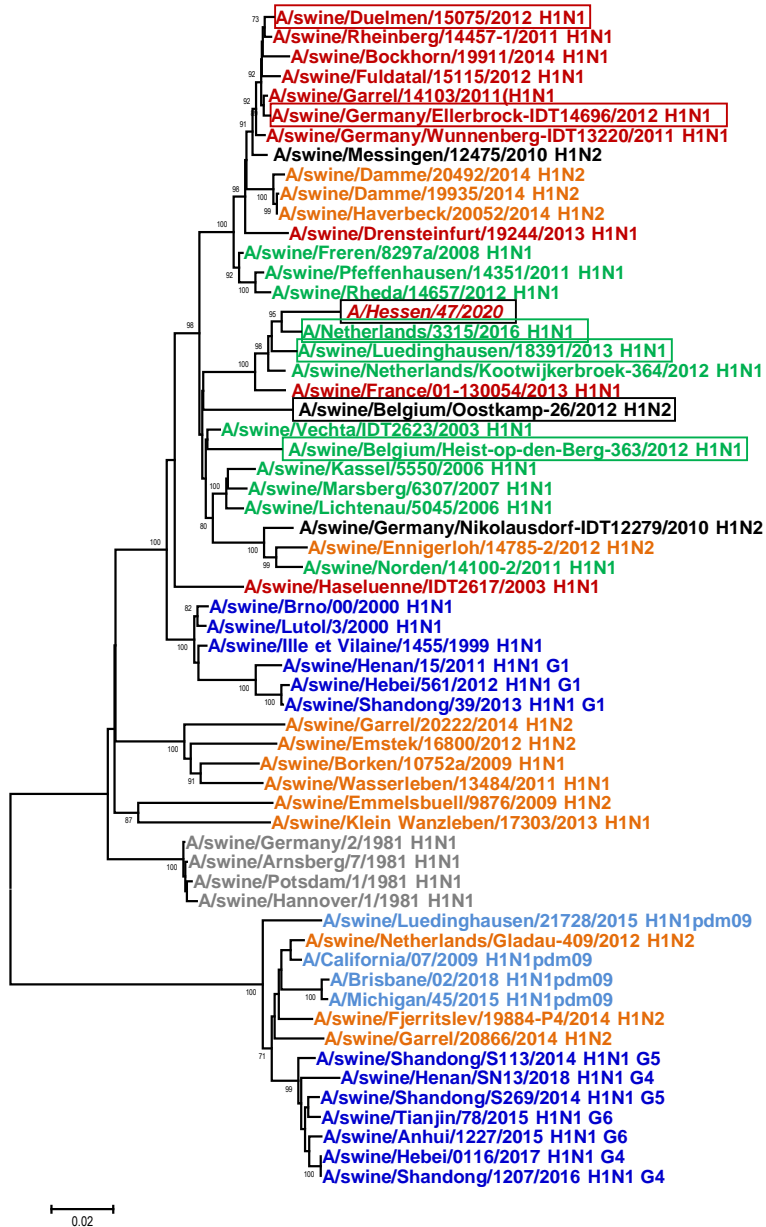


Figure S8: Phylogenetic analysis of PB1 gene of influenza A viruses. Virus genomes were analyzed by NGS and were phylogenetically evaluated with Mega7 (Neighbor-Joining method, midpoint rooted, bootstrap test with 1000 replicates, Kimura 2-parameter method, partial deletion (site coverage cutoff 5%). Sixty-one influenza A viruses were characterized: 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, Sun 2020 [3]). Closely related viruses that are identified by Blast analysis of each segment (data not shown) and used as reference viruses for further analysis, respectively, are framed: For HA, NA, swELLE/2012 and swDUEL/2012; for MP, NP, NS, NS1, PB1, PB1-F2, zoonotic NL/2016 + swLUED/2013; for PA, PA-X swHEIST/2012; and for PB2, swOOST/2012

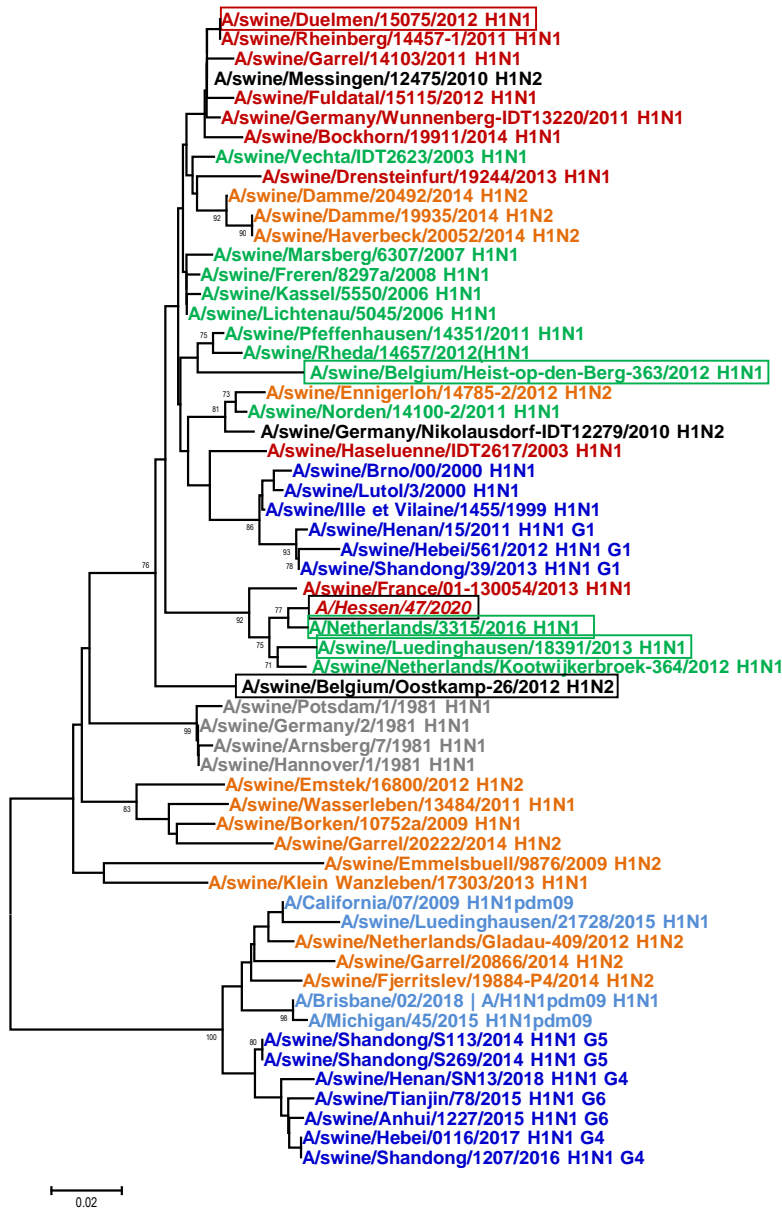


Figure S9: Phylogenetic analysis of PB1-F2 gene of influenza A viruses. Virus genomes were analyzed by NGS and were phylogenetically evaluated with Mega7 (Neighbor-Joining method, midpoint rooted, bootstrap test with 1000 replicates, Kimura 2-parameter method, partial deletion (site coverage cutoff 5%). Sixty-one influenza A viruses were characterized: 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, Sun 2020 [3]). Closely related viruses that are identified by Blast analysis of each segment (data not shown) and used as reference viruses for further analysis, respectively, are framed: For HA, NA, swELLE/2012 and swDUEL/2012; for MP, NP, NS, NS1, PB1, PB1-F2, zoonotic NL/2016 and swLUED/2013; for PA, PA-X swHEIST/2012; and for PB2, swOOST/2012

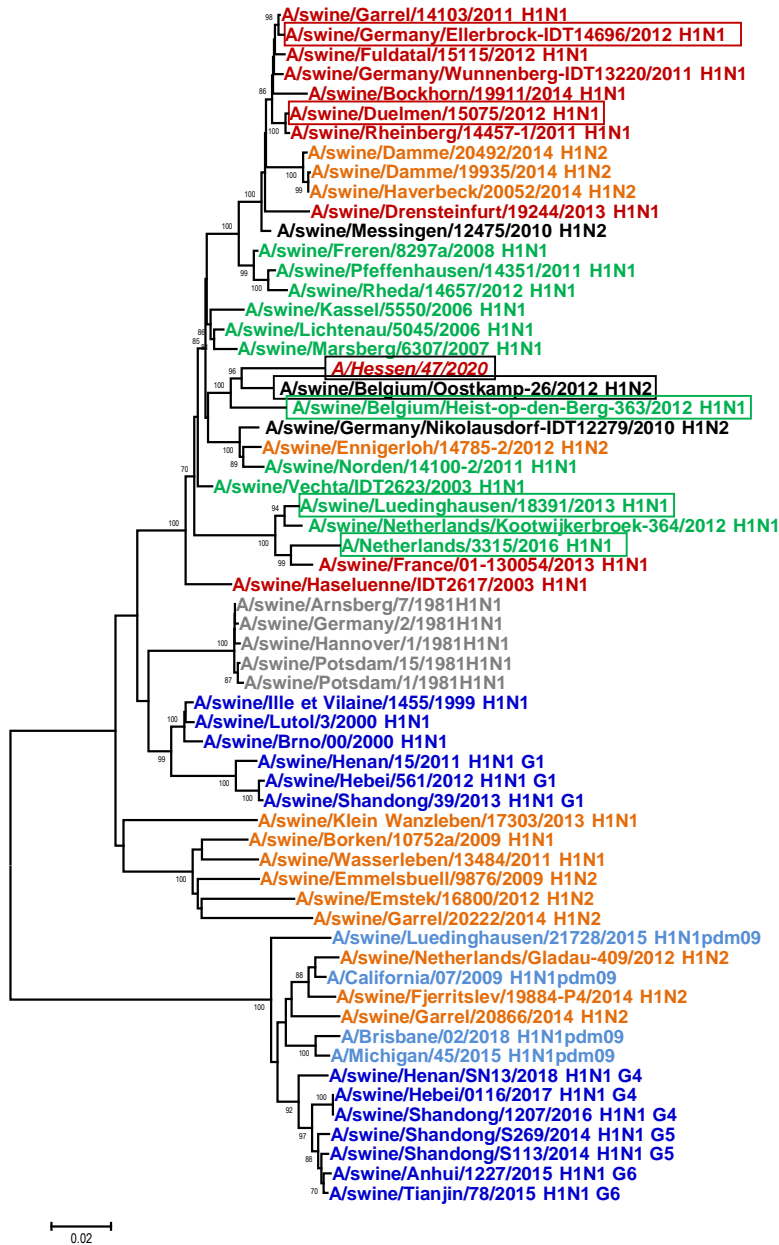


Figure S10: Phylogenetic analysis of PB2 gene of influenza A viruses. Virus genomes were analyzed by NGS and were phylogenetically evaluated with Mega7 (Neighbor-Joining method, midpoint rooted, bootstrap test with 1000 replicates, Kimura 2-parameter method, partial deletion (site coverage cutoff 5%). Sixty-one influenza A viruses were characterized: 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, Sun 2020 [3]). Closely related viruses that are identified by Blast analysis of each segment (data not shown) and used as reference viruses for further analysis, respectively, are framed: For HA, NA, swELLE/2012 and swDUEL/2012; for MP, NP, NS, NS-1, PB1, PB1-F2, zoonotic NL/2016 and swLUED/2013; for PA, PA-X swHEIST/2012; and for PB2, swOOST/2012

Table S1: Results of FluServer analysis (<https://flusurver.bii.a-star.edu.sg/>)

Query	All Mutations	Red Mutations	Orange Mutations	Reference
HA	A3V;R4K;L6F;L9F;A11S;A13T;T15L;N16K;I22V;V36I;D52T;K60S;K62N;I64K;G70K;K71N;I74L;L78I;S91N;S100P;N101D;E103K;D111E;I113A;S124T;K130R;T137A;S138T;E144D;K147R;V149T;A151I;Y155H;A156S;S159N;L168I;T169V;S173N;S179R;K180N;V183T;L193I;G202D;T203S;S207T;A212N;D213H;A214T;V216I;N224Y;R225Q;I231T;A232V;V237I;D239E;M244I;E252D;P253Q;R276K;G279S;T284M;D286N;P288R;D291N;N293T;K295M;I303L;N304K;S306N;I312V;V315I;R325K;K328H;Q382R;N387I;T393S;V399I;T408A;A409S;N416G;R419K;I435V;V444I;S457F;R460K;S468A;K471R;A491E;R497K;D502N;E516K;Y528H	S173N	L9F;S100P;D111E;K147R;A151I;A156S;S159N;T169V;A212N;N224Y;A232V;D239E;P253Q;D291N;N304K;S306N	HA A/BrevigMission/1/1918(H1N1)
MP	M248I			M1 A/California/07/2009(H1N1)
MP	T11I;C19Y;N20S;P25A;L26I;V27A;S31N;F48S;R77Q	L26I;V27A;S31N	F48S;R77Q	M2 A/Duck/Guangdong/E1/2012(H10N8)
NA	T9I;G11S;V13I;M15L;T16I;I17N;M19I;N21S;I23M;L40I;I46T;N59K;T72I;A75V;A76V;G77E;S79E;V81I;S82P;K84N;N88S;S90P;K111R;P126H;L140M;I188T;G210S;A232V;R257K;I263V;M269L;N270D;S286G;T289I;E311D;I321V;F322L;K331R;V338I;S340I;I365T;E398D;D416N;I418M;G454S		N88S;S90P;I365T	NA A/California/07/2009(H1N1)
NP	A22T;G34E;K48Q;R98K;R99K;E114D;M189L;A284I;A323V;R351K;V353I;Q357K;V363I;S377I;R384K;A423T;R452K;S482N;N498S		R99K;Q357K	NP A/Duck/HongKong/24/1976(H4N2)
NS	R21Q;D24N;Q25W;E26G;G47S;S48N;D53E;T56P;E66K;R67C;S73Y;M79I;I81T;A86T;R88H;M98I;V111L;A112T;S114P;I123V;D152E;V180I;G183K;T197A;S206T;D209I;L214F;P215I;P216T;A223E;V226I;E227G;S228P		I81T;P215I;A223E;V226I;E227G;S228P	NS1 A/Shearwater/Australia/2576/1979(H15N9)
NS	K18R;E26K;I31M;V49L;M52S;L55H;V83I;L120F		K18R;M52S	NS2 A/WSN//1933(H1N1)
PA	I13V;L42M;R57K;I61T;G66S;V94I;S184L;G186A;E206D;R212H;D216N;E252G;K262R;T263E;N321H;I323V;L335I;Q346N;M374V;V387I;E399K;P400V;I432V;I438V;I505V;L549I;A553S;T618V;T639A;P653S;E688D;T712M		R57K;P400V	PA A/Herringgull/NewJersey/780/1986(H16N3)
PB1	K52R;K54R;I69V;S152M;G154S;V191I;Q210H;R211K;T257A;S375D;I423V;R486K;I517V;T566M;R584H;E618V;Q621R;V632I;S633N;V640I;V645I;A648S;I682V;I735M		S375D	PB1 A/Duck/Alberta/60/1976(H12N5)
PB2	S12L;K61R;T76I;S107D;R251K;T271I;S286G;I292M;I295V;R299K;I338V;K353R;R389K;C409R;V457I;M473V;I478V;V480I;M483T;Q507P;V584I;D611E;M645L;V649I;D701N;K718R;I756L	D701N	I292M	PB2 A/Duck/Alberta/60/1976(H12N5)