H7N7 avian influenza virus mutation from low to high pathogenicity on a layer chicken farm in the UK

Supplementary Materials

Table S1. Immunohistochemical distribution of influenza A viral nucleoprotein antigen in carcasses from Sample Sets 1 and 3. Additional observations also included: N, necrosis; E, encephalitis; P, necrotizing pancreatitis and C, coelomitis/peritonitis – chronic active. N/A, not available; -, negative; +/-, minimal or rare antigen labelling; +, small number of labelled cells or antigen; ++, moderate antigen labelling; +++ abundant antigen labelling.

Sample Set	**1**		**3**																	
Shed	**10**	**12A**	**1**		**2**		**4**		**5**		**6**		**7**		**10**		**11**		**12A**	
Bird	**Mix**	**Mix**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
Heart	+	N/A	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-
Skin	-	N/A	-	-	N/A	N/A	-	-	-	-	-	-	-	-	-	N/A	-	N/A	-	-
Feather follicles	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	-	N/A	N/A	N/A
Skeletal muscle	-	N/A	-	-	-	-	-	-	-	-	-	-	-	N/A	-	-	-	-	-	-
Spleen	+++ N	+++ N	-	N/A	+++ N	+	-	-	-	-	+	-	-	-	+	-	+ N	+ N	+ N	-
Cecal Tonsil	+++	+	N/A	-	+	-	-	-	-	-	+	-	-	-	N/A	++	-	-	+	-
Brain	-	-	-	-	N/A	-	-	-	-	-	-	-	+/-	-	-	-	-	++ E	-	-
Kidney	++	+	-	-	N/A	-	-	-	-	-	-	-	-	-	-	-	-	+ N	+	-
Ovary	+	N/A	-	-	++	-	-	-	+	+	+	-	-	-	+	-	N/A	+	+ C	-
Oviduct		N/A	N/A	-	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	-	++	N/A	N/A	++	N/A	N/A
Lung	+	-	-	-	+	-	-	-	-	-	+	-	-	+/-	+	+	-	++	-	-
Trachea / Bronchi	++	+	-	-	N/A	N/A	-	-	-	-	+	-	-		+	-	-	-	N/A	-
Nasal turbinate	-	-	-	-	+++	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Air Sacs	+	+	N/A	N/A	N/A	N/A	-	-	+	N/A	+	-	-	+/-	N/A	N/A	N/A	N/A	N/A	-
Pancreas	N/A	N/A	-	-	+	-	-	-	-		-	-	-	-	+	-	-	++ P	-	-
Duodenum	+ C	N/A	-	-	+	-	-	-	-	-	+	-	-	-	N/A	-	-	-	-	-

Table S2 cont. Immunohistochemical distribution of influenza A viral nucleoprotein antigen in carcasses from Sample Sets 1 and 3. Additional observations also included: N, necrosis; E, encephalitis; P, necrotizing pancreatitis and C, coelomitis/peritonitis – chronic active. N/A, not available; -, negative; +/-, minimal or rare antigen labelling; +, small number of labelled cells or antigen; ++, moderate antigen labelling; +++ abundant antigen labelling.

Sample Set	**1**		**3**																	
Shed	**10**	**12A**	**1**		**2**		**4**		**5**		**6**		**7**		**10**		**11**		**12A**	
Bird	**Mix**	**Mix**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
Liver	+	+	-	-	++	-	-	-	-	-	-	-	-	-	-	-	-	N/A	++ N	-
Jejunum	++ C	+	-	-	+	-	-	-	-	-	-	-	-	-	+ C	-	-	+ C	+ C	-

Table S2. RRT-PCR and sequencing results from Sample Set 1. HA CS motifs for each shed were determined based on sequencing of pooled tissues. HP_G; HA CS motif PEIPRHRK**G**RGLF, HP_R; HA CS motif PEIPRHRK**R**RGLF, +; RRT-PCR positive, -; RRT-PCR negative, nt; not tested.

Shed	Type	Tissue	Tissue RRT-PCR Results				Cleavage Site Motif Detected	AIV Molecular Pathotype
			Influenza A	AOAV-1	H5	H7		
10	Free-range	Trachea (N=2)	+	-	-	+	nt	nt
10	Free-range	Cecal tonsil (N=2)	+	-	-	+	PEIPRHRK**G**RGLF	HP_G
12A	Caged	Trachea (N=4)	+	-	-	+	nt	nt
12A	Caged	Cecal tonsil (N=4)	+	-	-	+	PEIPRHRK**R**RGLF	HP_R
12A	Caged	Spleen (N=4)	+	-	-	+	nt	nt
12A	Caged	Proventriculus (N=1)	+	-	-	+	nt	nt

Changes in the HA CS motif are shown in bold.

Table S3. Summary of the carcass tissues from Sample Set 3 with RRT-PCR results. HA CS motifs for each shed were determined based on sequencing of individual swabs. HP_G; HA CS motif PEIPRHRK**G**RGLF, HP_R; HA CS motif PEIPRHRK**R**RGLF, LP; HA CS motif PEIPKGRGLF, +; RRT-PCR positive, -; RRT-PCR negative.

Shed	Type	AIV Molecular Pathotype	Tissue RRT-PCR Results (2 carcasses per shed)			
			Brain	Lung and Trachea	Viscera	Intestine
1	Caged	LP/HP_R	-	-	-	-
2	Caged	LP/HP_R	+	+	+	+
4	Free-range	LP	-	-	-	-
5	Free-range	HP_G/HP_R	-	-	-	-
6	Free-range	HP_G/HP_R	-	+	+	+
7	Free-range	HP_G/HP_R	-	-	-	-
10	Free-range	HP_G/HP_R	-	+	+	+
11	Free-range	HP_R	+	+	+	+

Table S4. Summary of the type isolate viruses obtained from each shed in Sample Set 2.

Shed	Type	Name of virus isolate
1	Caged	A/chicken/England/26350/2015
2	Caged	A/chicken/England/26352/2015
10	Free-range	A/chicken/England/26346/2015
11	Free-range	A/chicken/England/26348/2015
12A	Caged	A/chicken/England/26354/2015

Table S5. Polymorphisms observed in A/chicken/England/26352/2015 that may confer adaptation to mammalian species or altered susceptibility to existing antivirals.

Protein	Amino Acid Position/Motif[1]	Phenotypic Consequences	Reference
PB2	Collective mutations: Leu89Val, Gly309Asp, Thr339Lys, Arg477Gly, IIe495Val, Lys627Glu, Ala676Thr	Enhanced polymerase activity and increased virulence in mice	[48]
PB1-F2	Asn66Ser	Increased virulence, replication efficiency and antivirus response in mice	[49-51]
HA[2]	Ser128Ala	Increased binding to human receptors	[52]
HA[2]	Thr151Ala	Loss of glycosylation increased binding to human receptors	[52]
HA[2]	Gly177Val	Increased binding to human receptors	[52]
NA[3]	His274Arg	Reduced susceptibility to oseltamivir and peramivir	[53-59]
M1	Asn30Asp	Increased virulence in mice	[60]
M1	Thr215Ala	Increased virulence in mice	[61, 62]
NS1	Ile101Met	Increased virulence in mice	[63, 64]
NS1	222-225 (presence of PDZ ligand domain)	Increased virulence in mice	[65]

Reference for Table S5.

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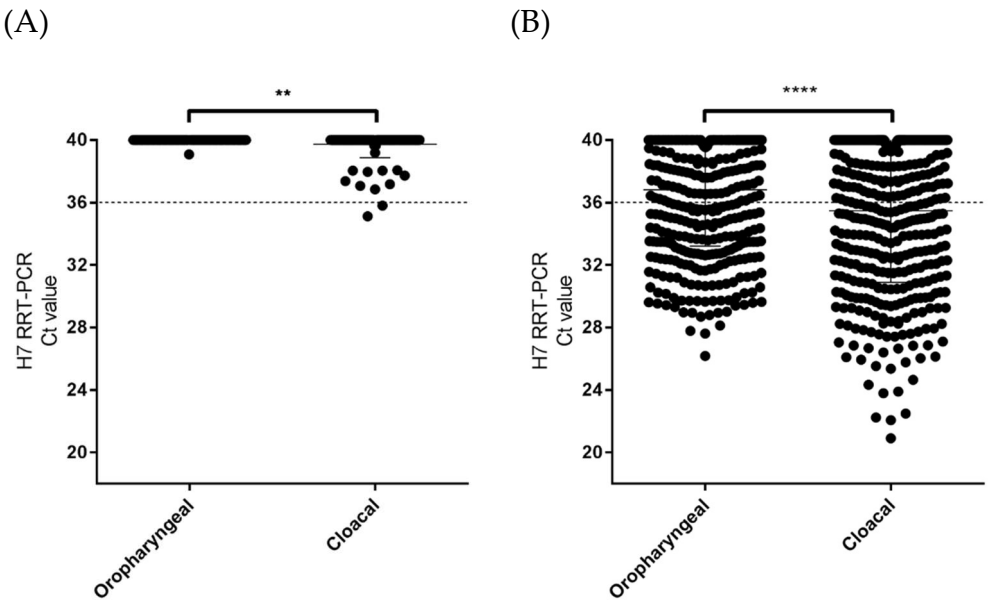
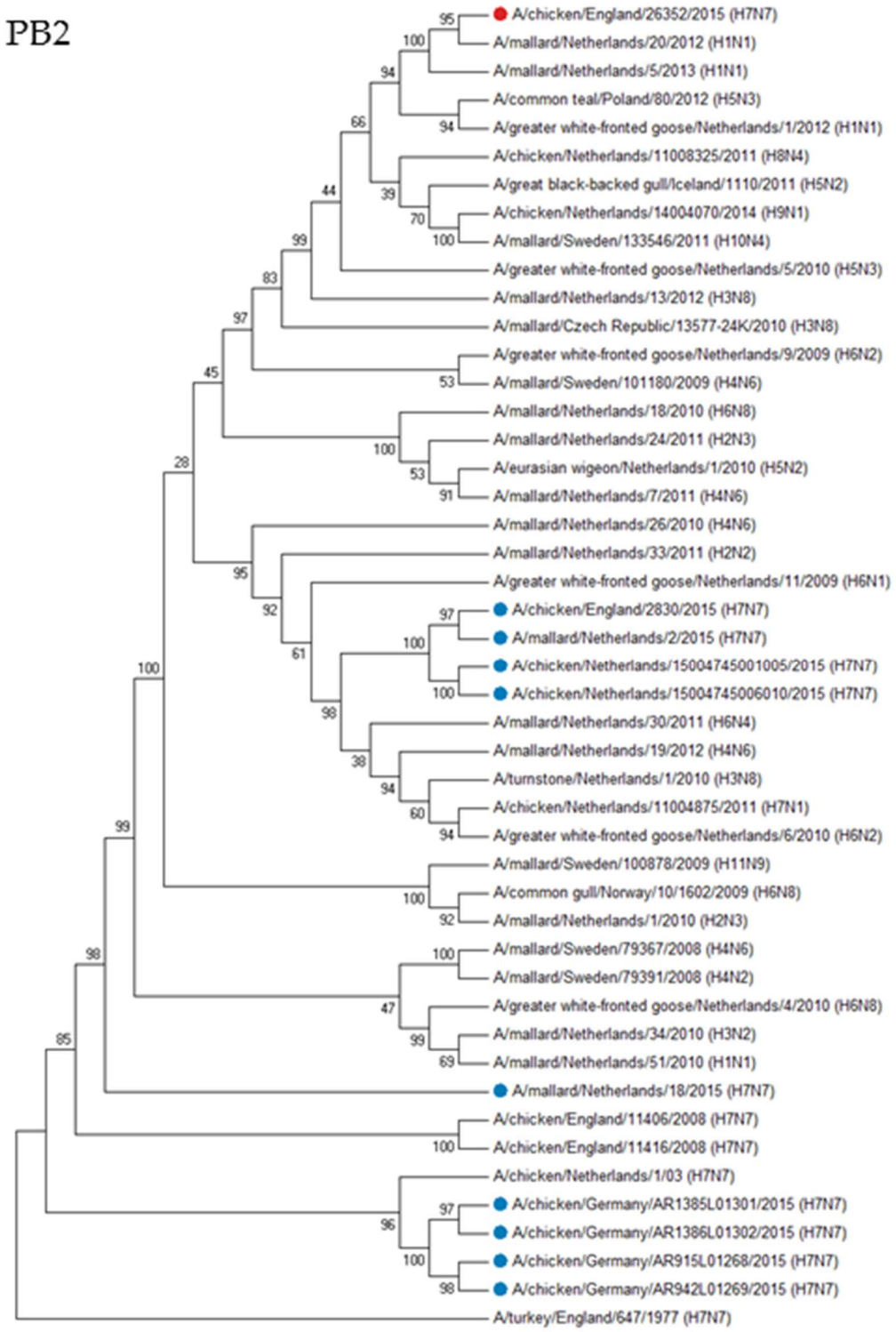
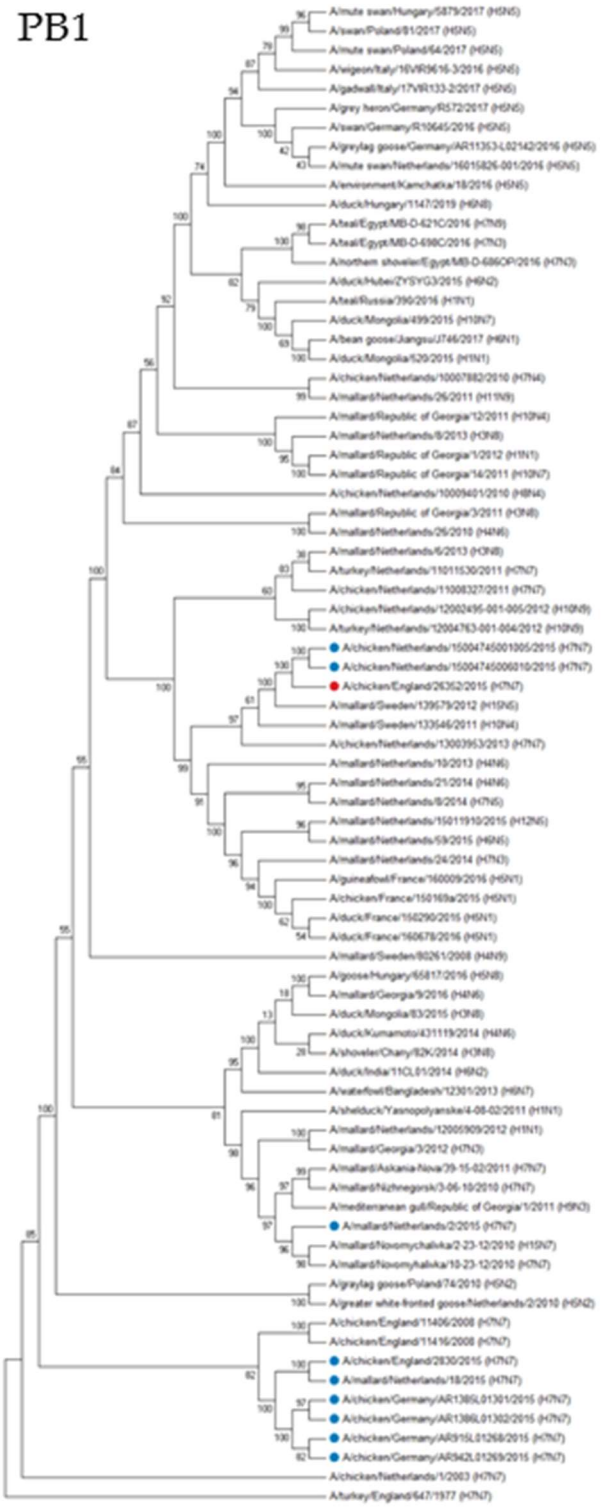


Figure S1. Previous UK H7N7 outbreaks were also shed via the gastrointestinal route. OP and cloacal swabs collected during **(A)** the H7N7 LPAIV outbreak in February 2015 [3] (N=120, per swab type) and **(B)** a H7N7 HPAIV outbreak in 2008 [4] (N=441, per swab type) were tested by H7 RRT-PCR and the level of H7 RNA compared using a Paired T-test. ** $p<0.01$ and **** $p<0.0001$.

(A)


PB2
A/chicken/England/26352/2015 (H7N7)
A/mallard/Netherlands/20/2012 (H1N1)
A/mallard/Netherlands/5/2013 (H1N1)
A/common teal/Poland/80/2012 (H5N3)
A/greater white-fronted goose/Netherlands/1/2012 (H1N1)
A/chicken/Netherlands/11008325/2011 (H8N4)
A/great black-backed gull/Iceland/1110/2011 (H5N2)
A/chicken/Netherlands/14004070/2014 (H9N1)
A/mallard/Sweden/133546/2011 (H10N4)
A/greater white-fronted goose/Netherlands/5/2010 (H5N3)
A/mallard/Netherlands/13/2012 (H3N8)
A/mallard/Czech Republic/13577-24K/2010 (H3N8)
A/greater white-fronted goose/Netherlands/9/2009 (H6N2)
A/mallard/Sweden/101180/2009 (H4N6)
A/mallard/Netherlands/18/2010 (H6N8)
A/mallard/Netherlands/24/2011 (H2N3)
A/eurasian wigeon/Netherlands/1/2010 (H5N2)
A/mallard/Netherlands/7/2011 (H4N6)
A/mallard/Netherlands/26/2010 (H4N6)
A/mallard/Netherlands/33/2011 (H2N2)
A/greater white-fronted goose/Netherlands/11/2009 (H6N1)
A/chicken/England/2830/2015 (H7N7)
A/mallard/Netherlands/2/2015 (H7N7)
A/chicken/Netherlands/15004745001005/2015 (H7N7)
A/chicken/Netherlands/15004745006010/2015 (H7N7)
A/mallard/Netherlands/30/2011 (H6N4)
A/mallard/Netherlands/19/2012 (H4N6)
A/turnstone/Netherlands/1/2010 (H3N8)
A/chicken/Netherlands/11004875/2011 (H7N1)
A/greater white-fronted goose/Netherlands/6/2010 (H6N2)
A/mallard/Sweden/100878/2009 (H11N9)
A/common gull/Norway/10/1602/2009 (H6N8)
A/mallard/Netherlands/1/2010 (H2N3)
A/mallard/Sweden/79367/2008 (H4N6)
A/mallard/Sweden/79391/2008 (H4N2)
A/greater white-fronted goose/Netherlands/4/2010 (H6N8)
A/mallard/Netherlands/34/2010 (H3N2)
A/mallard/Netherlands/51/2010 (H1N1)
A/mallard/Netherlands/18/2015 (H7N7)
A/chicken/England/11406/2008 (H7N7)
A/chicken/England/11416/2008 (H7N7)
A/chicken/Netherlands/1/03 (H7N7)
A/chicken/Germany/AR1385L01301/2015 (H7N7)
A/chicken/Germany/AR1386L01302/2015 (H7N7)
A/chicken/Germany/AR915L01268/2015 (H7N7)
A/chicken/Germany/AR942L01269/2015 (H7N7)
A/turkey/England/647/1977 (H7N7)

(B)


PB1
A/mute swan/Hungary/5879/2017 (H5N5)
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A/wigeon/Italy/16VIR9616-3/2016 (H5N5)
A/gadwall/Italy/17VIR133-2/2017 (H5N5)
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A/swan/Germany/R10645/2016 (H5N5)
A/greylag goose/Germany/AR11353-L02142/2016 (H5N5)
A/mute swan/Netherlands/16015826-001/2016 (H5N5)
A/environment/Kamchatka/18/2016 (H5N5)
A/duck/Hungary/1147/2019 (H6N8)
A/teal/Egypt/MB-D-621C/2016 (H7N9)
A/teal/Egypt/MB-D-698C/2016 (H7N3)
A/northern shoveler/Egypt/MB-D-686OP/2016 (H7N3)
A/duck/Hubei/ZYSYG3/2015 (H9N2)
A/teal/Russia/390/2016 (H1N1)
A/duck/Mongolia/499/2015 (H10N7)
A/bean goose/Jiangsu/J746/2017 (H6N1)
A/duck/Mongolia/529/2015 (H1N1)
A/chicken/Netherlands/10007882/2010 (H7N4)
A/mallard/Netherlands/26/2011 (H11N9)
A/mallard/Republic of Georgia/12/2011 (H10N4)
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A/mallard/Republic of Georgia/1/2012 (H1N1)
A/mallard/Republic of Georgia/14/2011 (H10N7)
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A/mallard/Republic of Georgia/3/2011 (H3N8)
A/mallard/Netherlands/26/2010 (H4N6)
A/mallard/Netherlands/6/2013 (H3N8)
A/turkey/Netherlands/11011530/2011 (H7N7)
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A/mallard/Sweden/139579/2012 (H10N8)
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A/chicken/Netherlands/13003953/2013 (H7N7)
A/mallard/Netherlands/10/2013 (H4N6)
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A/mallard/Netherlands/8/2014 (H7N5)
A/mallard/Netherlands/15011910/2015 (H12N5)
A/mallard/Netherlands/59/2015 (H6N5)
A/mallard/Netherlands/24/2014 (H7N3)
A/guineafowl/France/160009/2016 (H5N1)
A/chicken/France/150169a/2015 (H5N1)
A/duck/France/150290/2015 (H5N1)
A/duck/France/160678/2016 (H5N1)
A/mallard/Sweden/80261/2008 (H4N9)
A/goose/Hungary/65817/2016 (H5N8)
A/mallard/Georgia/9/2016 (H4N6)
A/duck/Mongolia/83/2015 (H3N8)
A/duck/Kumamoto/431119/2014 (H4N6)
A/shoveler/Chany/82K/2014 (H3N8)
A/duck/India/11CL01/2014 (H9N2)
A/waterfowl/Bangladesh/12301/2013 (H9N7)
A/shelduck/Yasnopolyanska/4-08-02/2011 (H1N1)
A/mallard/Netherlands/12005909/2012 (H1N1)
A/mallard/Georgia/3/2012 (H7N3)
A/mallard/Askania-Nova/39-15-02/2011 (H7N7)
A/mallard/Nizhnegorsk/3-06-10/2010 (H7N7)
A/mediterranean gull/Republic of Georgia/1/2011 (H9N3)
A/mallard/Netherlands/2/2015 (H7N7)
A/mallard/Novomychalivka/2-23-12/2010 (H15N7)
A/mallard/Novomykhalivka/10-23-12/2010 (H7N7)
A/greylag goose/Poland/74/2010 (H9N2)
A/greater white-fronted goose/Netherlands/2/2010 (H9N2)
A/chicken/England/11406/2008 (H7N7)
A/chicken/England/11416/2008 (H7N7)
A/chicken/England/2830/2015 (H7N7)
A/mallard/Netherlands/18/2015 (H7N7)
A/chicken/Germany/AR1385L01301/2015 (H7N7)
A/chicken/Germany/AR1386L01302/2015 (H7N7)
A/chicken/Germany/AR915L01268/2015 (H7N7)
A/chicken/Germany/AR942L01269/2015 (H7N7)
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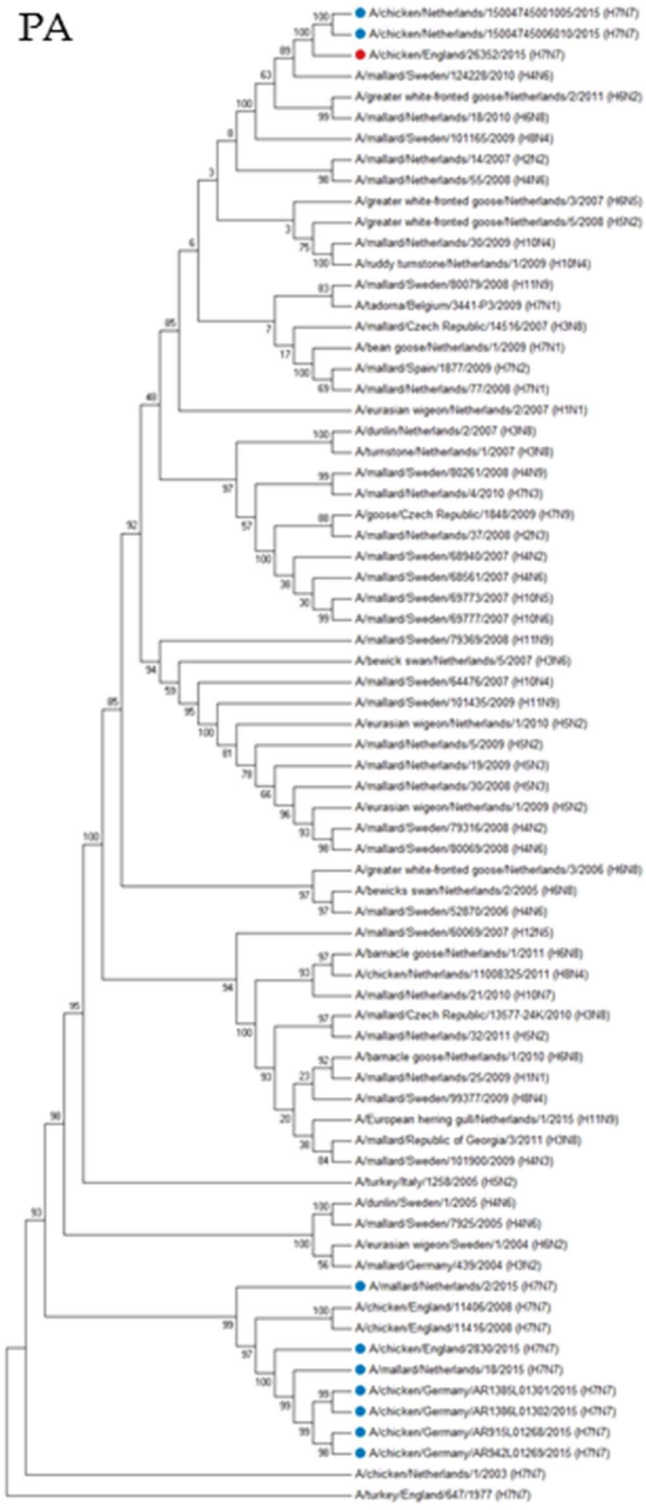
A/turkey/England/647/1977 (H7N7)

(C)


PA
A/chicken/Netherlands/15004745001005/2015 (H7N7)
A/chicken/Netherlands/15004745006010/2015 (H7N7)
A/chicken/England/26352/2015 (H7N7)
A/mallard/Sweden/124228/2010 (H4N6)
A/greater white-fronted goose/Netherlands/2/2011 (H6N2)
A/mallard/Netherlands/18/2010 (H6N8)
A/mallard/Sweden/101165/2009 (H8N4)
A/mallard/Netherlands/14/2007 (H2N2)
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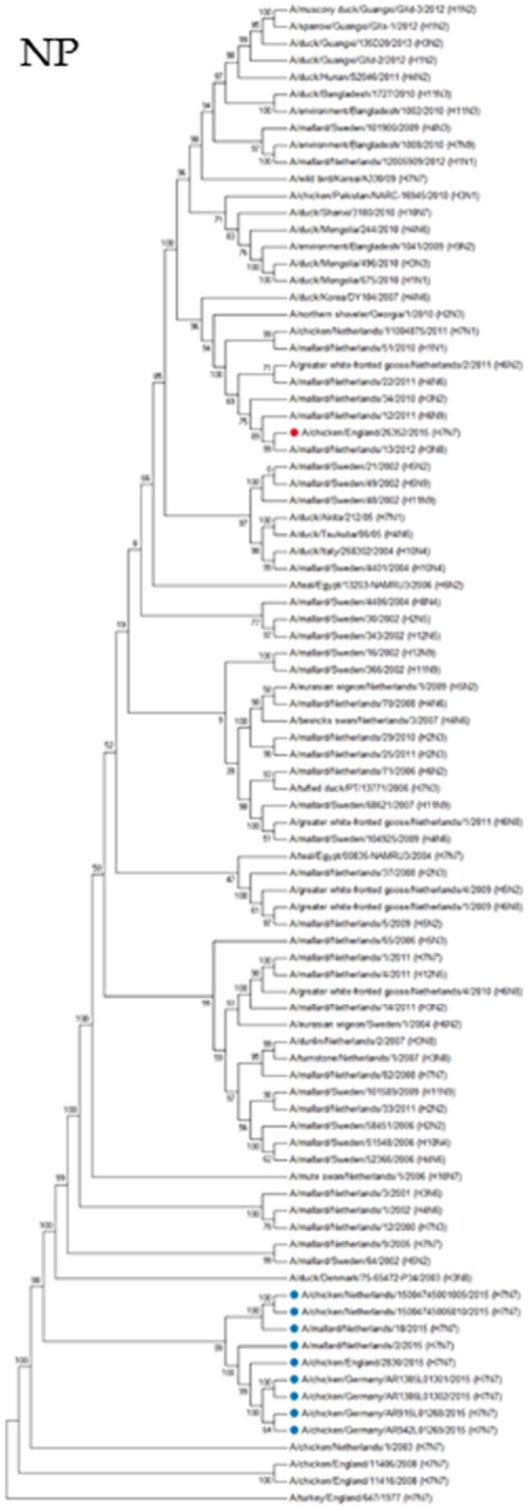
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A/mallard/Sweden/16/2002 (H12N9)
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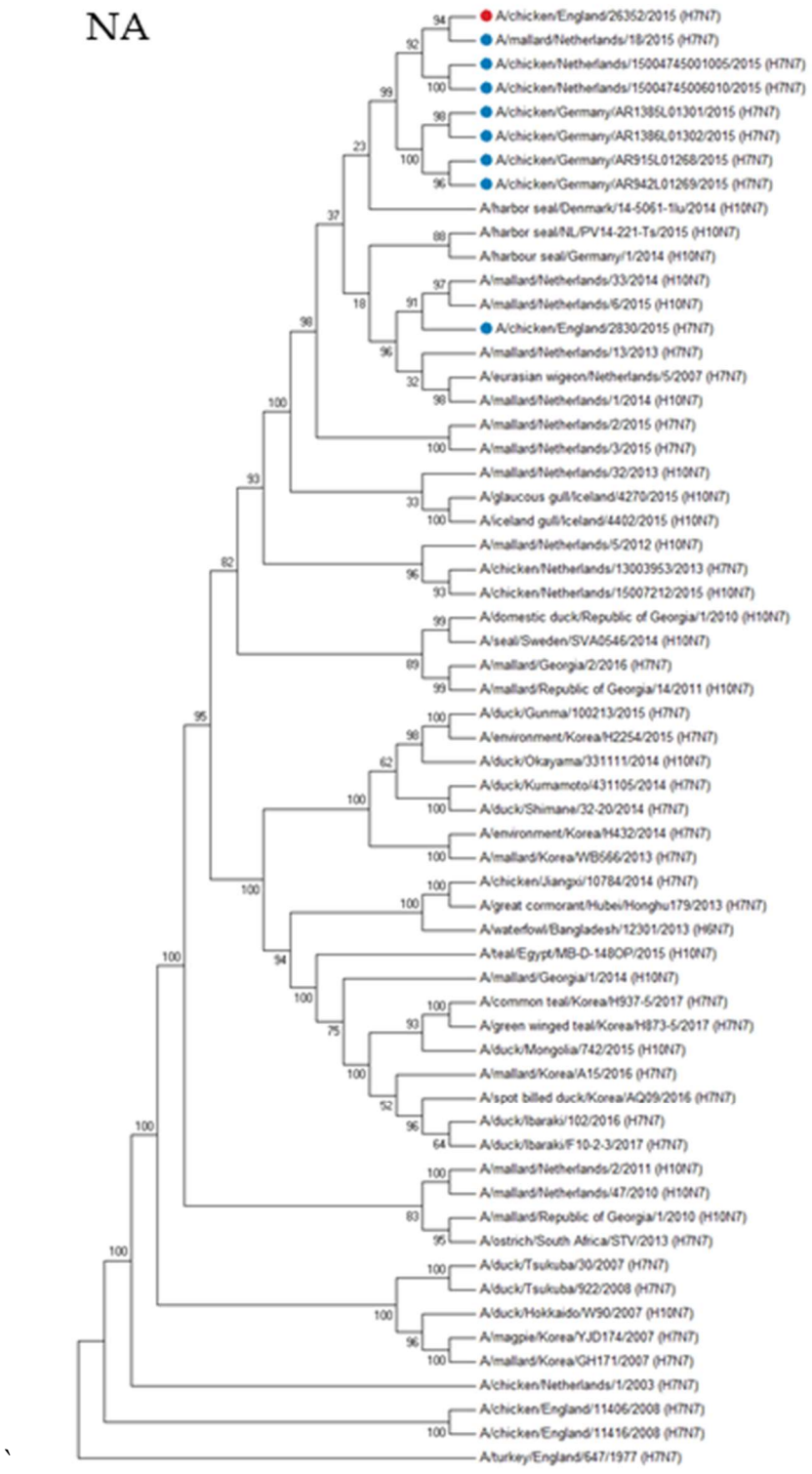
(E)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A/green winged teal/Korea/H873-5/2017 (H7N7)
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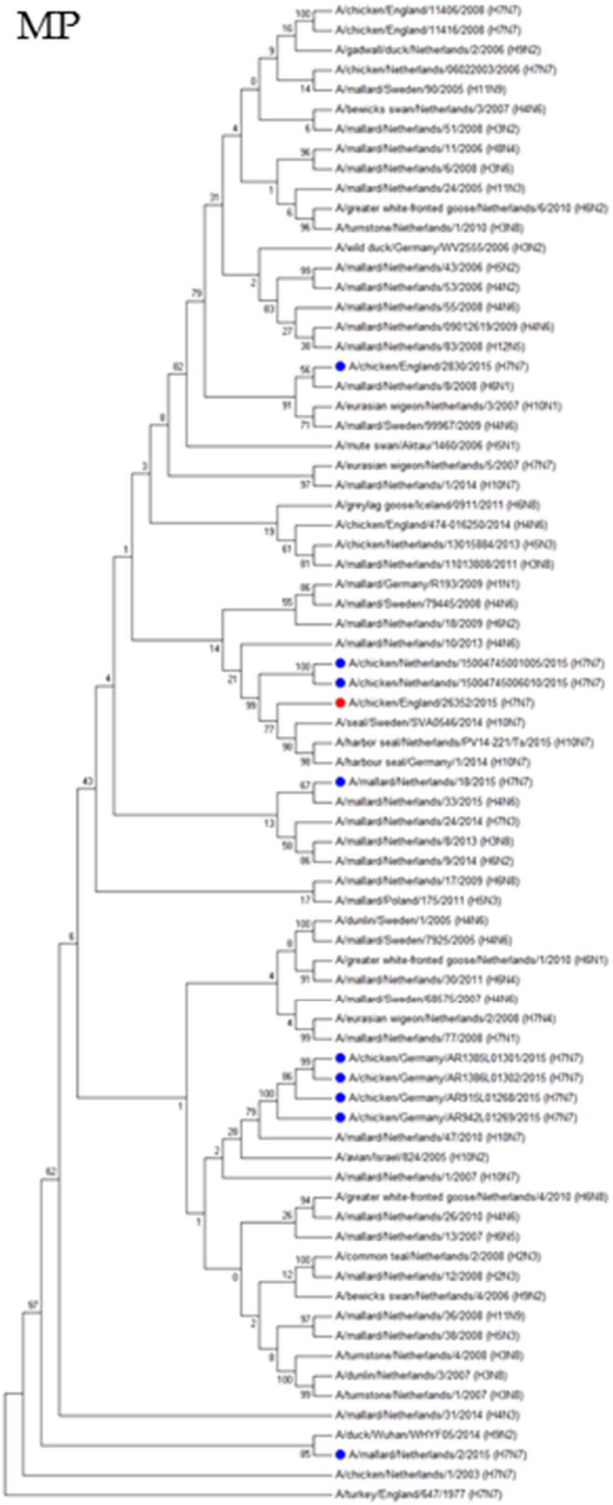
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A/mallard/Netherlands/24/2005 (H11N3)
A/greater white-fronted goose/Netherlands/6/2010 (H9N2)
A/turnstone/Netherlands/1/2010 (H3N8)
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A/mallard/Sweden/7925/2005 (H4N6)
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A/greater white-fronted goose/Netherlands/4/2010 (H6N8)
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A/mallard/Netherlands/12/2008 (H2N3)
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A/mallard/Netherlands/36/2008 (H11N9)
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A/dunlin/Netherlands/3/2007 (H3N8)
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(G)

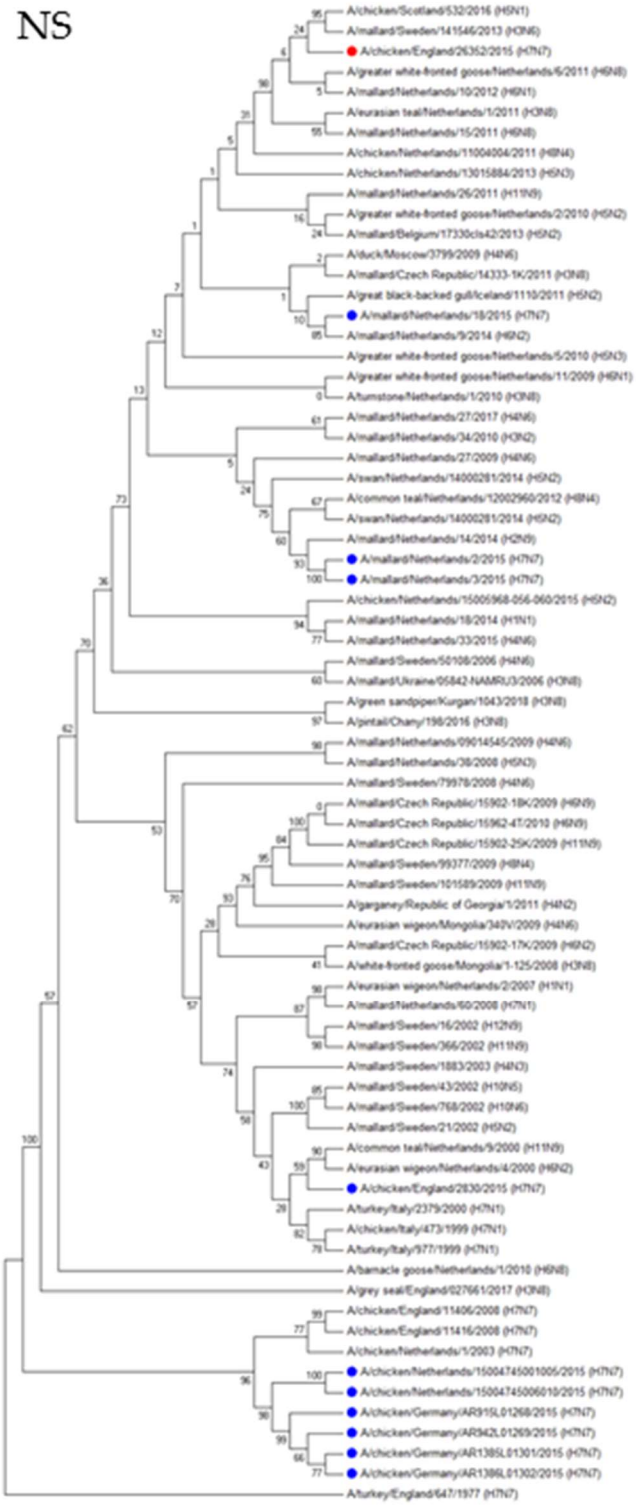
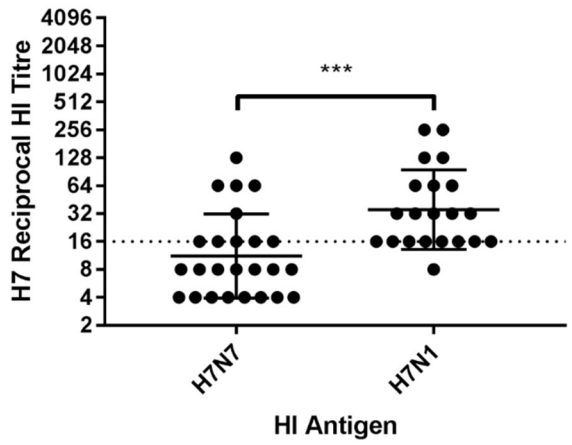


Figure S2. A/chicken/England/26352/2015 is related to AIVs from wild birds. Phylogenetic analysis was conducted using a maximum-likelihood approach for the following influenza A genes: (**A**) PB2, based on the TIM+F+G4 model; (**B**) PB1, based on the TIM+F+G4 model; (**C**) PA, based on the TVM+F+G4 model; (**D**) NP, based on the TVM+F+I+G4 model; (**E**) NA, based on the K3PU+F+G4 model; (**F**) M, based on the K3P+I model and (**G**) NS, based on the HKY+F+I model. A/chicken/England/26352/2015 (H7N7) is indicated by a red circle, whilst contemporary European H7N7 influenza viruses from 2015 are indicated by blue circles. Suitable models were determined using IQ-Tree [17] with ModelFinder [18].

(A)



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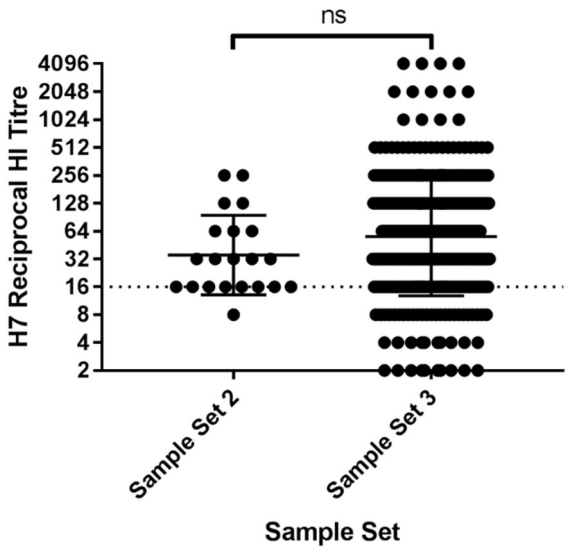

Figure S3. Sera from the outbreak demonstrated greater affinity to the H7N1 AIV antigen. (**A**) Sera from Sample Set 2 were tested by HI against the H7N7 (N=84) and H7N1 (N=21) AIV antigens. The reciprocal HI titers towards these antigens were compared using a Mann-Whitney test. (**B**) The reciprocal HI titers towards the H7N1 AIV antigen of sera from Sample Sets 2 (N=21) and 3 (N=531) were compared using a Mann-Whitney test. ns, not significant $p>0.05$, *** $p<0.001$, **** $p<0.0001$. Graphs show geometric mean ± geometric SD.