

# Analysis of influenza A viruses of subtype H1 from wild birds, turkeys and pigs in Germany reveals interspecies transmission events

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**Background** Despite considerable host species barriers, interspecies transmissions of influenza A viruses between wild birds, poultry and pigs have been demonstrated repeatedly. In particular, viruses of the subtypes H1 and H3 were transmitted between pigs and poultry, predominantly turkeys, in regions with a high population density of both species. The recovery of a swine influenza H1N1 virus from a turkey flock in Germany in 2009 prompted us to investigate molecularly the subtype H1 viruses recently detected in wild birds, pigs and poultry.

**Objectives** The goal of this study was to investigate the relationship between H1N1 viruses originating from wild and domestic animals of Germany and to identify potential trans-species transmission or reassortment events.

**Methods** Hemagglutinin and neuraminidase gene or full-length genome sequences were generated from selected, current H1N1 viruses from wild birds, pigs and turkeys. Phylogenetic analyses were combined with genotyping and analyses of the deduced

amino acid sequences with respect to biologically active sites. Antigenic relationships were assessed by hemagglutination inhibition reactions.

**Results** Phylogenetic analysis of the hemagglutinin sequences showed that viruses from distinct H1 subgroups co-circulate among domestic animals and wild birds. In addition, these viruses comprised different genotypes and were distinguishable antigenically. An H1N1 virus isolated from a turkey farm in northern Germany in 2009 showed the highest similarity with the avian-like porcine H1N1 influenza viruses circulating in Europe since the late 1970s.

**Conclusions** The data demonstrate the genetic and antigenic heterogeneity of H1 viruses currently circulating in domestic and wild animals in Germany and points to turkeys as a possible bridge between avian and mammalian hosts.

**Keywords** Animal influenza A viruses, diversity, H1N1, phylogeny.

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## Introduction

Wild aquatic birds of the orders *Anseriformes* and *Charadriiformes* constitute a reservoir in which all subtypes of influenza A viruses are perpetuated. Because of the genetic and phenotypic variability of these viruses, incidental transmission of these viruses can cause infections in animal species outside the natural reservoir, including domestic poultry, mammalian animal species and humans. Presumably, most of such transmission events remain unnoticed, because of hampered viral replication and a lack of clinical signs. Occasionally, however, the transmitted viruses are successful at adapting to their new host species, and a new stable virus lineage, which can then evolve independently from its progenitor(s) in the previous natural host reservoir, is established.<sup>1</sup> Such events have often been

reported in pigs, horses, dogs and humans.<sup>2–5</sup> In Europe, wild bird H1N1 viruses were transmitted to pigs in the late 1970s, establishing a stable lineage, and displacing the ‘classical’, 1918-descendant swine H1N1 virus. Once viruses of avian origin have adapted to a mammalian host, inter-mammalian transmission (e.g. pigs-humans, horses-dogs) is facilitated.<sup>6–8</sup> Despite intense research, it remains difficult to predict which specific polygenetic changes are required for successful influenza virus interspecies transmission.<sup>9</sup>

Interspecies transmission of influenza virus is dependent on both host and viral genetic factors. Several barriers blocking virus transmission between birds and mammals function at different levels of the viral replication cycle.<sup>10</sup> They involve interactions of the viral envelope glycoproteins, hemagglutinin (HA) and neuraminidase (NA), with specific glycan receptors, that differ among individual

species in both structure and tissue distribution,<sup>11,12</sup> as well as in the recruitment of cellular factors required for efficient replication.<sup>13</sup> Finally, the virus has to deal with species-specific variations in the innate immune system, as well as humoral and cellular immunity.<sup>2,14,15</sup>

Among the 16 different HA subtypes identified to date, viruses of the H1 and the H3 subtypes, in particular, have repeatedly overcome species barriers and established several distinguishable lineages, which are currently co-circulating in humans, pigs, horses, and poultry,<sup>16</sup> and are distinct from the avian H1 and H3 clades maintained in the natural host reservoir.

Reverse modes of influenza virus transmission from mammals to birds have also been reported.<sup>17–20</sup> These are of particular interest, as these viruses have apparently acquired a broadened host range, and thus are able (after adaptation to a mammalian species) to re-infect birds. Interspecies transmissions of viruses of subtypes H1 and H3 between pigs and poultry have predominantly involved turkeys, which appear to be more susceptible to swine influenza viruses than other species of domestic poultry.<sup>21</sup> The first serological evidence of swine influenza viruses of subtype H1N1 in turkeys was obtained in 1975.<sup>22</sup> The recent introduction of a swine-origin influenza virus (S-OIV) of subtype H1N1 in Mexico and the USA in humans and its subsequent pandemic spread are consistent with this notion of a broadened host range of some H1 viruses. This virus has undergone multiple reassortant events and harbours genome segments originating from Eurasian and American swine influenza virus lineages.<sup>23</sup> In addition, the virus has been repeatedly transmitted by infected patients to other mammalian and avian species, including pigs, cats, pet ferrets and turkeys,<sup>24–26</sup> which also has been documented under experimental conditions.<sup>27–30</sup>

To investigate the diversity of subtype H1 viruses detected recently in poultry, swine and wild birds in Germany, we performed phylogenetic analyses of sequences derived from different regions and compared these with sequence data obtained from GenBank (NCBI Influenza Virus Research Database; <http://www.ncbi.nlm.nih.gov/genomes/FLU/Database/nph-select.cgi?go=database>). The results reveal that several clusters of subtype H1 viruses co-circulate in domestic animals and wild birds, with continued individual exchange of lineages between different host species. In support of this, a recent influenza virus isolate of subtype H1N1 from a turkey farm in Germany was shown to be entirely derived from a swine H1N1 virus strain.

## Material and methods

### Origin of influenza virus samples

Samples from wild birds in Germany (usually combined oropharyngeal and cloacal swabs) were collected within the

context of an EU-coordinated wild bird monitoring programme. Poultry swab samples were obtained from routine monitoring or targeted sampling in clinically suspicious flocks. These also included samples from a farm where more than 39 000 fattening turkeys were kept in close proximity to a pig fattening facility. No other poultry species were present. No clinical signs, increased mortality or virological or serological evidence for an influenza infection were found during the previous routine inspection of the turkey farm, about 4 weeks before the collection of the H1-positive samples began. Porcine samples consisted of nasal swabs or lung tissue from pigs with respiratory disease.

Serological investigations, virus isolation and RT-PCR on these samples were performed at the OIE and National Reference Laboratory for Avian Influenza and the Laboratory for Swine Influenza at the Friedrich-Loeffler-Institut, according to recommended and standardised protocols (EU decision 437/2006).

### Virus isolation and characterisation

Virus isolation from avian material was attempted using embryonated hen eggs, as prescribed in the EU AI Diagnostic Manual. Porcine samples were processed for virus isolation in MDCK cells as previously described.<sup>27</sup>

Virus subtyping was accomplished serologically using a hemagglutination inhibition (HI) assay, by sequence analysis of the HA gene<sup>31</sup> and using conventional NA subtype-specific RT-PCR<sup>32</sup> or micro array analyses.<sup>33</sup>

### Amplification and sequencing of viral nucleic acids

RNA extraction, RT-PCR assays and sequence analysis of full-length genome segments were carried out as described.<sup>34</sup> Sequences of additional primers required to amplify and sequence the H1 genome segments of recent European swine influenza viruses (in three overlapping parts) are given in Table 1.

### Phylogenetic analyses

Except those established in this study, sequences for phylogenetic analysis were retrieved from the GenBank database, aligned with MAFFT,<sup>35</sup> and trimmed to equal lengths using BioEdit.<sup>36</sup> Alignments then were further analysed using TopAli v2.5.<sup>37</sup> A General Time Reversible model with rate heterogeneity and a proportion of invariant sites was selected (ModelSelection) for PhyML analysis using 100 bootstrap runs. Analyses of the H1 HA genes included 242 full-length sequences and focused mainly on those of avian and pig origin. Human seasonal influenza sequences have been omitted from the alignment for clarity. The final maximum likelihood (ML)-tree comprised 175 sequences (Figure 1A). The clade numbering system used is as described in Liu *et al.*<sup>38</sup> NA sequences were analysed in a similar manner. The final

**Table 1.** Primer pairs used for the overlapping amplification and sequence analysis of swine influenza A virus subtype H1 HA gene segments

Amplicon	New primer	Sequence (5'-3')	Established primer	Amplicon position
H1-1	pigH1-951R	GGATGTAYRTTCTGRAAWGGGAG	BmHA-F*	nt 1-951
H1-2	pigH1-595F	AARGAAGTSCYGTMRMHTGG	PHIPPS-1110R**	nt 595-1110
H1-3	pigH1-951F	CTYCCWTTYCAGAAVRTACATCC	BmNS-890R*	Nt 951-17

\*Hoffmann *et al.*, (2001); \*\*Phipps *et al.*, 2004.

NA tree contained 141 N1 sequences of human seasonal and pandemic H1N1, American and Eurasian avian HxN1, porcine H1N1 and 'classical' porcine H1N1 viruses and H5N1 highly pathogenic avian influenza virus (HPAIV) (Figure 1B). Topologies established using either a neighbour-joining or a parsimony approach yield essentially the same clustering and, therefore, are not shown here.

## Results

### Detection of influenza A viruses of subtype H1 in wild birds

Influenza A viruses of subtype H1 which have been isolated from wild birds in Germany are summarised in Table 2. Additional H1 viruses were detected by rRT-PCR assays, including sequencing of a short HA-part for subtyping (Table 2). No signs of disease have been recorded in wild birds in which infections with subtype H1 influenza A viruses were detected. The HA and NA sequences of five of the isolated wild bird viruses, as well as the complete genomic sequence of one, were established (Table 3). Phylogenetic analyses of the HA coding gene group sequences from wild birds in Germany closely related to other contemporary avian isolates from Europe and Asia of subgroup H1-1.2 (Figure 1A). Sequences of viruses detected from 2006 to 2008 diverged considerably from those originating in 2009 and clustered in different sister clades. Even more pronounced, the NA sequences of wild bird H1N1 viruses were found to be widely separated in the large cluster of Eurasian sequences, although the isolates analysed originated from a restricted geographical region and were collected over a period of only 4 years (Figure 1B). Analysis of the antigenic profile of the avian isolates by HI (Table 4) revealed that they form a common group, recognised by hyperimmune serum raised against AIV A/ wild duck/ Germany/R30/06, but not by sera raised against swine influenza viruses or the pandemic (H1N1) 2009 virus.

### Detection of influenza viruses of subtype H1 in pigs

No systematic surveillance of swine influenza A viruses was conducted in the context of this study. Instead, a cross-

section of several previous isolates, and a selection of more recent and current isolates from different regions of Germany were included in the analyses. Two isolates from 2009 were fully sequenced, and for eight additional isolates the HA and NA coding genes were sequenced (Table 3). The HA coding genes of pig-derived viruses, in contrast to those of wild bird H1 viruses, formed a group of closely related sequences in cluster H1-1.3 (Figure 1A). Similarly, all the N1 gene sequences of pig viruses established in this study clustered closely together in a group of Eurasian virus sequences (Figure 1B). Regarding the antigenic profile of swine influenza viruses, it is interesting to note that sera from pigs infected with H1N1 isolates from 2009 react with all swine-derived H1N1 isolates from this period but do not show cross-reactivity to isolates from 2000/2001 or pandemic H1N1. However, when using hyperimmune serum raised against A/swine/Belzig/2/2001 (H1N1), reactivity broadens and includes pandemic H1N1/2009 virus as well as the historic human isolate A/FM/1/47. In contrast, serum raised against A/swine/Bakum/1832/2000 (H1N2) reacts (in addition to the homologous antigen) significantly only with isolate A/FM/1/47.

### Detection of influenza viruses of subtype H1 in turkeys

Influenza A virus H1N1 in turkeys was detected in routine sampling material from a commercial farm situated in northeast of Germany. Real-time PCR yielded Ct values between 22 and 26 in the majority of oropharyngeal swabs sampled, indicating substantial virus replication within individual birds and a high prevalence in the flock.

As shown in Figure 1(A, B), there is a strong phylogenetic correlation between sequences from the turkey isolate R778/09 (purple labelling in Figure 1) derived from this outbreak, and the porcine sequences SIV-leipzig11308/09 and SIV-R1773/09 isolated from swine in Germany in the same year. In addition, nucleotide sequence analysis of the remaining six gene segments identified SIV-R1737/09 as the most homologous virus when compared to the turkey sequences, and prominent differences with respect to sequences obtained from wild birds (>98% similarity compared to pig sequences versus <90% similarity compared to



**Figure 1.** Phylogenetic trees of influenza virus HA segment subtype 1 (A) and NA segment subtype 1 (B) generated by a maximum likelihood approach. Branches are drawn in proportion to the scale bar. Numbers at nodes represent bootstrap values after 100 replications (for clarity only numbers >90 are shown). Sequences shown in red were generated in this study. Turkey-derived sequences representing an interspecies transmission event from pigs in Germany are shown in green. Sequences labelled blue point out other presumed interspecies transmission events between avian and porcine hosts.

sequences from the most recent wild bird virus R193/09). Furthermore, analysis by genotyping<sup>39</sup> revealed identical genotypes (F, G, I, 1C, F, 1F, F, 1E) for the turkey virus (R778/09) and the completely sequenced swine viruses. However, lineage prediction for the turkey PA must be handled carefully, because only <75% of the sequence is

available. In contrast, three segments (PB2, PA and NA) of the H1N1 genomic sequence of the wild mallard isolate R193/09 belonged to different genotypes (G, G, D, 1C, F, 1K, F, 1E). Although the remaining five wild bird-derived genome segments belong to the same genotype as the swine viruses, they clearly cluster only with avian sequences from

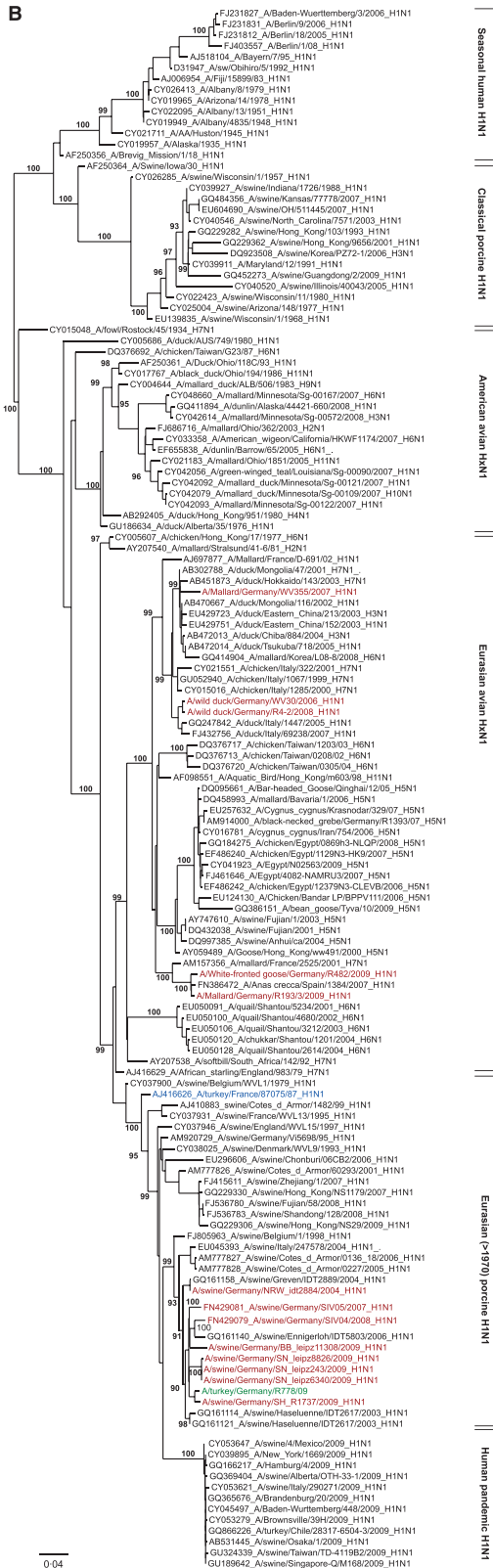


Figure 1b. (Continued).

wild birds and poultry with >95% similarity (data not shown). As such, the turkey H1N1 isolate, R778/09, represents a direct interspecies transmission of a virus of purely porcine origin.

### Analysis of individual gene regions presumably involved in virus transmission

#### Putative HA antigenic sites

Among the 13 putative antigenic sites identified in the HA of subtype H1 viruses,<sup>40</sup> the turkey H1N1 virus revealed swine virus amino acid (aa) signatures at 11 sites. Two of these antigenic sites were unique for the turkey sequence and the sequence of SIV-leipzig11308/09 (aa 53-58 TSHNGK and aa 99-102 TSDS; numbering from the start codon M of the coding region) compared to the other swine sequences. Sequences from wild bird viruses analysed here differed at 7 of the 13 sites when compared to the majority of swine virus sequences, while two of the sites were shared by SIV-vi5698/95.

#### Receptor-binding sites

In addition, the putative 11 amino acids involved in forming the receptor-binding site<sup>40</sup> were identical in turkey R778/09 and SIV-leipzig11308/09, whereas the wild bird virus sequences differed at five positions. Three of these five positions (aa 151, 169, 239) were again identical in the five wild bird viruses and SIV-vi5698/95.

#### Potential N-linked glycosylation sites

Six potential N-linked glycosylation sites were identified in the deduced HA amino acid sequences for pig (with the exception of SIV-leipzig8826/09), turkey and wild bird virus sequences (NetNGlyc 1.0 Server). All isolates sequenced in this study shared potential N-glycosylation sites at positions 28 (NST), 40 (NVT), 498 (NGT) and 557 (NGS). Wild bird virus sequences possess additional potential sites at positions 104 (NGT) and 304 (NSS), whereas sequences of viruses from pigs (except SIV-leipzig8826/09) and turkey show two further potential sites at positions 212 (NHT or NPT) and 291 (NCT). Interestingly, pig SIV-vi5698/95 again clustered with the wild bird viruses instead of showing the swine virus pattern. The most probable glycosylation sites *in vivo* are at positions 28, 40, 304 and 557 for wild bird viruses, and 28, 40 and 557 for pig and turkey viruses (potential score ++ or higher; see <http://www.cbs.dtu.dk/services/NetNGlyc/>).

#### Other genome segments

Taking into consideration the sequences of all eight segments of the turkey and the closest respective pig virus, a total of 49 exchanges were found at the amino acid level, unevenly distributed across the segments. A comparatively

**Table 2.** Detection of subtype H1 influenza viruses by real-time RT PCR and virus isolation in wild birds in Germany

Year	Reference	Species	Federal State	Material	Isolate
2006	R30	Wild duck	NW	Cloacal swab	A/wild duck/Germany/R30/06
	R1419	Egyptian goose	NW	Cloacal swab	A/Egyptian goose/Germany/R1419/06
2007	R355	Mallard	NI	Tissues	A/mallard duck/Germany/R355/07
	R1649	Mallard	HE	Cloacal swab	None
	R2067	Tufted duck	BY	Cloacal swab	None
	R2068	Mallard	BY	Cloacal swab	None
	R2719	Mallard	BY	Combined swab	None
	R2897	Mallard	BY	Cloacal swab	None
	R3036	Mallard	NW	Cloacal swab	A/mallard duck/Germany/R3036/07
	R3038	Mallard	BW	Cloacal swab	None
	R3240	Mallard	MV	Cloacal swab	None
2008	R4	Mallard	NW	Combined swab	A/mallard/Germany/R4/08
	R120	Mallard	ST	Cloacal swab	None
	R234	Mallard	BY	Combined swab	None
	R292	Mallard	NW	Combined swab	A/mallard/Germany/R292/08
2009	R193	Mallard	RP	Combined swab	A/mallard/Germany/R193/09
	R481	White-fronted goose	NI	Cloacal swab	A/white fronted goose/Germany/R481/09
	R482	White-fronted goose	NI	Cloacal swab	A/white fronted goose/Germany/R482/09
	R491	Mallard	BY	Combined swab	None

Abbreviations for German Federal States: NW, North Rhine Westfalia; NI, Lower Saxony; HE, Hesse; BY, Bavaria; MV, Mecklenburg-West-Pomerania; ST, Saxony-Anhalt; RP, Rhineland Palatinate.

All viruses belonged to NA subtype N1.

high proportion of amino acid exchanges are present in the M2 gene (9 aa, 3%), the NS1 gene (5 aa, 2.2%) and the HA gene (14 aa, 2.5%). Although no information is available about the biological relevance of these different amino acids with respect to the whole genome, these exchanges include sites in proteins responsible for forming ribonucleoprotein complexes and viral polymerases.

## Discussion

Wild birds were actively monitored in the context of different European Union research programmes. Viruses of the HA subtype H1 have been detected continuously in wild bird populations in Germany since 2006. H1N1 strains circulating among pig populations of Europe since the late 1970s are representatives of the historically avian-derived lineage of porcine H1 viruses. H1 infections appear to be widespread in pigs in some central European countries, including Germany, with up to 80% seroprevalence in sow populations.<sup>41</sup> However, a few of these viruses (mainly those originating from France, Italy, Spain and Germany) have been characterised molecularly. H1 virus infections in poultry have been reported only sporadically in European countries (e.g. Italy, France). The reason for this might be not only underreporting because of mild clinical signs or completely subclinical infections, but also a

lack of H1 subtype-specific serological surveillance. The last reported cases worldwide originated in Germany in 1990 (turkey),<sup>42</sup> the USA in 1999 (turkey),<sup>43</sup> Italy in 2003–2007 (duck and goose),<sup>44</sup> France in 2005 (turkey)<sup>45</sup> and China in 2005 (duck) (Liu J, Bi Y, Fu G, Yang J, Feng J, Ma G, Liu Q, Pu J and Tian F, unpublished data; Accession Number FJ536843).

Phylogenetic analyses of H1 and N1 sequences of viruses from wild birds, pigs and poultry revealed a deeply branched tree for the HA sequences. In the NA tree, three clusters can be distinguished: (i) seasonal human H1N1 isolates, which are linked to a large subcluster of so-called 'classical' H1N1 swine viruses still circulating in the United States and Asia, (ii) American avian isolates and (iii) Eurasian avian isolates. The latter also harbours the N1 of the HPAI H5N1 virus (marked with blue branches in Figure 1B) and the so-called 'avian-like' lineage of swine H1N1 viruses. Linked to these viruses are N1 gene segments also found in the new pandemic human H1N1. In the H1 tree, sequences of viruses isolated from several poultry species, which were located in sub-clusters of swine virus sequences, are marked (blue), and in the N1 tree, one turkey sequence from France was found to be clustered among swine sequences. These phylogenies might point to direct trans-species transmission or reassortment events. Wild bird subtype H1N1 viruses detected in Germany in recent years (for which HA and NA genes have been

**Table 3.** Summary of full-length (genome segments) sequences created in this study

Host origin	Isolate	Genome segment											
		HA	NA	PB1	PB2	PA	NP	M	NS				
Porcine	A/swine/Germany/V5698/1995	AM920728	AM920729	/	/	/	AM920730	AM920731	AM920732	/	AM920733	AM920734	AM920741
	A/swine/Germany/Sek1178/2000	AM920737	AM920738	/	/	/	AM920739	AM920740	AM920741	/	/	/	/
	A/swine/Germany-NW/IDT2889/04	EPI248509	EPI248508	/	/	/	/	/	/	/	/	/	/
	A/swine/Germany/SIV05/2007	FN429080	FN429081	/	/	/	FN429082	FN429083	FN429084	/	/	/	/
	A/swine/Germany/SIV07/2008	FN429078	FN429079	/	/	/	/	/	/	/	/	/	/
	A/swine/Germany-SN/siv-leipz243/09	EPI248503	EPI248502	/	/	/	/	/	/	/	/	/	/
	A/swine/Germany-SN/siv-leipz6340/09	EPI248505	EPI248504	/	/	/	/	/	/	/	/	/	/
	A/swine/Germany-SN/siv-leipz8826/09	EPI248507	EPI248506	/	/	/	/	/	/	/	/	/	/
	A/swine/Germany-BB/siv-leipz11308/09	EPI248493	EPI248492	EPI248491	EPI248490	EPI248489	EPI248486	EPI248488	EPI248487	/	/	/	/
	A/swine/Germany-SH/R1737/09	EPI248485	EPI248484	EPI248483	EPI248482	EPI248481	EPI248478	EPI248480	EPI248479	/	/	/	/
	A/wild duck/Germany/VW30/06	EPI248514	EPI248512	/	/	/	EPI248513	EPI248511	EPI248510	/	/	/	/
	A/wild duck/Germany/VW355/07	EPI248519	EPI248517	/	/	/	EPI248518	EPI248516	EPI248515	/	/	/	/
	A/wild duck/Germany-NW/R04/08	EPI248521	EPI248520	/	/	/	/	/	/	/	/	/	/
	A/mallard/Germany-RP/R193/09	EPI248501	EPI248500	EPI248499	EPI248498	EPI248497	EPI248494	EPI248496	EPI248495	/	/	/	/
A/white-front goose/Germany-NI/R482/09	EPI248525	EPI248524	/	/	/	/	/	/	/	/	/	/	
A/turkey/Germany-NI/R778/09	EPI248477	EPI248476	EPI248475	EPI248474	EPI248473	EPI248470	EPI248472	EPI248471	/	/	/	/	

sequenced) were found to cluster together in both the H1 and the N1 trees in 'wild bird lineages' formed by other Eurasian wild bird virus sequences. Within these sequence clusters, the HA sequences from Germany were located in sister clades, according to their year of isolation (2006–2008 versus 2009). This result is mirrored by the NA phylogeny, indicating the introduction of H1N1 viruses with a new variant set of surface glycoprotein genes. Although the isolates analysed originated from a restricted geographical region and were collected over only 4 years, this could be attributed to either a reassortment event or an ongoing adaptive evolution of the virus.

Phylogenetic analyses of both the HA and the NA sequences, as well as genotyping of the full genome sequence, match the turkey virus sequences directly to one of the swine virus sequences obtained in Germany in 2009. These results, and the circumstantial epidemiologic evidence that the samples were collected from turkeys on a farm situated close to a pig fattening facility, suggest direct transmission of the porcine virus to turkeys without any reassortment. The spread of swine influenza H1N1 viruses to turkeys is not a new finding and has been previously reported in the United States and Europe.<sup>17,42,43</sup> Infections with both 'classical' H1N1 and 'avian-like' swine influenza, as well as the more recent reassortant H1N1 and H3N2 swine influenza viruses in turkeys have been reported.<sup>44</sup> However, only two of the above-mentioned cases have been phylogenetically correlated with direct transmission of H1N1 virus from pigs to poultry (A/turkey/Germany/2482/90,<sup>42</sup> and Figure 1; A/duck/Hebei/843/2005, Liu *et al.*, 2009, unpublished data; Accession Number FJ536843). In most cases of swine influenza virus transmission to turkeys, the turkey flocks were located in close proximity to pigs. The preference of swine-origin influenza viruses for turkeys over other poultry species might be attributed to special features in the upper respiratory tracts of turkeys: Yassine *et al.*<sup>46</sup> has demonstrated the presence of substantial amounts of NeuAc $\alpha$ -2,6Gal receptors in the trachea of turkeys, which would explain the ability of swine viruses to infect the upper respiratory tract of turkeys. Nevertheless, the effect of primarily spatial links between turkey and pig fattening farms (both of which can contain large concentrations of animals) cannot be excluded.

Full-length genome sequence analysis of an H1N1 virus isolated from a turkey farm in northern Germany in 2009 confirmed the swine origin of this virus. In total, six genotypes have been identified in Eurasian swine influenza H1N1 viruses, and three have been documented for avian H1N1. The genotype confirmed for the turkey virus, R778/09, represents the predominant genotype for pig Eurasian H1N1 viruses which emerged first in France in 1984 and were last documented in Germany in 2004 (A/swine/Greven/

**Table 4.** Serological cross-reactivity of various influenza A H1 virus isolates (HI assay)

Antigen	Antiserum <sup>1</sup>						
	H1N1/ FM/1/ 47 <sup>2*</sup>	H1N1/ Regensburg/ 09 <sup>3**</sup>	H1N1/SW/ Belzig/2/ 01 <sup>3*</sup>	H1N2/SW/ Bakum/ 1832/00 <sup>3*</sup>	H1N1/SW/ DE-SN/8826/ 09 <sup>3**</sup>	H1N1/SW/ DE-TH/R2241/ 09 <sup>3**</sup>	H1N1/duck/ DE/R30/06 <sup>4*</sup>
A/FM/1/47	<b>8.3</b>	<4.3	6.3	8.3	<4.3	<4.3	<4.3
A/California/7/09 (H1N1)	<4.3	<b>9.3</b>	5.3	4.3	<4.3	<4.3	4.3
A/Bavaria/74/09 (H1N1)	<4.3	<b>8.3</b>	7.3	<4.3	<4.3	<4.3	5.3
A/SW/Belzig/2/01 (H1N1)	<4.3	<4.3	<b>10.3</b>	4.3	<4.3	<4.3	6.3
A/SW/Bakum/1832/00 (H1N2)	4.3	<4.3	4.3	<b>11.3</b>	<4.3	<4.3	<4.3
A/SW/DE-SN/8826/09 (H1N1)	<4.3	5.3	10.3	5.3	<b>6.3</b>	7.3	5.3
A/SW/DE-TH/R2241/09 (H1N1)	<4.3	4.3	10.3	4.3	5.3	<b>6.3</b>	4.3
A/SW/DE-BB/11308/09 (H1N1)	<4.3	4.3	10.3	4.3	4.3	5.3	5.3
A/dk/DE/1/77 (H1N1)	5.3	<4.3	5.3	4.3	<4.3	<4.3	6.3
A/duck/DE/R30/06 (H1N1)	<4.3	<4.3	4.3	<4.3	<4.3	<4.3	<b>5.3</b>

■, homologous antigen/antiserum; \*, hyperimmune sera; \*\*, sera from convalescent pigs, 3 weeks p.i.; □, phylogenetically closely related IVA to A/tk/DE/R778/09, where no virus isolate is available.

□Rabbit: 1 HI-titre (log<sub>2</sub>) of RDE treated sera; 2 serum; 3 pig serum; 4 chicken serum.

IDT2889/2004). Amino acid pattern analysis of the receptor-binding sites of the turkey and swine virus HA sequences documented no differences between them. In conclusion, no species-specific adaptation appears to have occurred in the HA as a consequence of interspecies transmission, although clear differences exist when comparing receptor binding sites of porcine versus wild bird H1 viruses.

The results presented in this work provide molecular evidence for direct interspecies transmission of porcine H1N1 influenza A virus to turkeys. Similarly, reports from the USA have documented frequent exchanges of H3 subtype viruses between pigs, turkeys and humans. Based on these findings, it may be hypothesised that, in addition to pigs, turkeys are involved in the maintenance and evolution of influenza A viruses with broadened transmission potential. In the light of natural and experimental infections of turkeys with the new pandemic H1N1 virus (presumably of swine origin), a new and menacing reassortment scenario emerges in areas such as Egypt or Southeast Asia, where the pandemic H1N1 virus and the highly pathogenic avian H5N1 influenza virus co-circulate. Lessons from the most recent pandemic suggest that more attention must be paid to the detection and characterisation of swine influenza viruses, even in non-porcine species. An enquiring eye should also be kept on turkeys as a possible entry port and mixing vessel for avian and swine influenza viruses.

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