

Genetic analysis of highly pathogenic Avian Influenza A (HPAI) H5N1 viruses isolated from Turkey between 2005 and 2008

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Summary: An outbreak of Highly Pathogenic Avian Influenza A (HPAI) H5N1 infection was first reported on 07 October 2005 in Turkey. There was a single outbreak in free range turkeys in the west side of the country on the Aegean coast in Kızıksa Village of Manyas District of Balıkesir Province. Following this outbreak, there were new outbreaks in different regions of Turkey respectively in December 2005, 2006, 2007 and 2008.

This study provides a characterization of HPAI H5N1 viruses isolated from Turkey that caused between 2005 and 2008. A phylogenetic analysis of the hemagglutinin gene showed that the Turkish HPAI H5N1 isolates were genetically closely related to the Asian lineage.

Key words: Avian Influenza Viruses, H5N1, diagnosis, Turkey

Türkiye’de 2005-2008 yılları arasında izole edilen kuş gribi viruslarının genetik analizi

Özet: Türkiye’de Yüksek Patojen Kuş Gribi H5N1 enfeksiyonu ilk olarak 07 Ekim 2005 tarihinde rapor edildi. Hastalık Balıkesir ili Manyas ilçesi Kızıksa Köyünde bir hindi çiftliğinde tek bir vaka olarak şekillendi. Bu vakayı takiben 2005, 2006, 2007 ve 2008 yıllarında Türkiye’nin değişik bölgelerinde yeni vakalar şekillendi.

Bu çalışmada 2005 ve 2008 yılları arasında Türkiye’de izole edilen Yüksek Patojen Kuş Gribi H5N1 viruslarının karakterizasyonları yapılmıştır. Bu izolatların Haemagglutinin geninin kısmi filogenetik analizi genetik olarak Asya’da bulunanlar ile yakın ilişkide olduğunu göstermektedir.

Anahtar sözcükler: Kuş Gribi Virusları, H5N1, Teşhis, Türkiye

Introduction

HPAI (H5N1) virus infection was announced in 1996 in China. HPAI (H5N1) virus is isolated from a farmed goose in Guangdong Province, China in 1996. Human infections with HPAI (H5N1) are reported in Hong Kong in 1997. Research on viruses isolated from dead birds in Qinghai Lake in 2005 demonstrates transmission of the virus among migratory geese and suggests that the virus may be carried along winter migratory routes. Disease spread out to everywhere year to year and some human cases occurred (Table 1)

(www.who.int/csr/disease/avian_influenza/Timeline_08_12_08.pdf).

The disease was first reported on 07 October 2005 in Turkey. There was a single outbreak in free range turkeys in the west side of the country on the Aegean coast in Kızıksa Village of Manyas District of Balıkesir Province. The outbreak was successfully controlled without any local or long-distance spread. This isolate (DQ407519) was sequenced and analysed by VLA Weybridge (BROWN, 2006).

Table 1. Distribution of human cases of HPAI (H5N1) between December 2003 and April 2006 (WHO, 2006).

Country	Onset of first reported case	Onset of last reported case	Number of cases	Number of deaths
Viet Nam	December 2003	November 2005	91	42
Thailand	January 2004	November 2005	22	14
Cambodia	January 2005	March 2006	6	6
Indonesia	July 2005	March 2006	32	24
China	October 2005	April 2006	18	12

Turkey	December 2005	January 2006	12	4
Iraq	January 2006	January 2006	2	2
Azerbaijan	February 2006	March 2006	8	5
Egypt	March 2006	April 2006	12	4
Total			203	113

Following the initial outbreak, a second outbreak was confirmed in domestic poultry on 25 December 2005, in the east of the country, Aralık that is in the district of Iğdir Province. Between the dates of 25 December 2005 and 31 March 2006, Turkey had 230 confirmed cases of Avian Influenza, 229 of which were of HPAI (H5N1) virus and 1 of which was of Low Pathogenic Avian Influenza (LPAI) H7N1 virus. Amongst these outbreaks, 200 outbreaks had occurred in domestic poultry and 30 cases were in wildbirds. Also, some human avian influenza infection occurred in 2006; this human case was one of the first confirmed reports of outside Asia and Africa.

Similar outbreaks occurred in neighboring countries of Turkey and Europe (Table 2) (www.who.int/csr/disease/avian_influenza/Timeline_08_12_08.pdf).

Outbreaks of HPAI H5N1 have been reported for wildlife and poultry in Turkey and neighbouring countries since 2005. A phylogenetic analysis of the hemagglutinin gene showed that the HPAI H5N1 isolates from Turkey were of avian origin and contained the hemagglutinin gene of the Asian lineage. The virus isolates in the EU and the neighbouring countries appear to be genetically closely related to the Asian lineage of the virus that has been isolated in China (Qinghai Lake), Russia (Southern Siberia) and Mongolia.

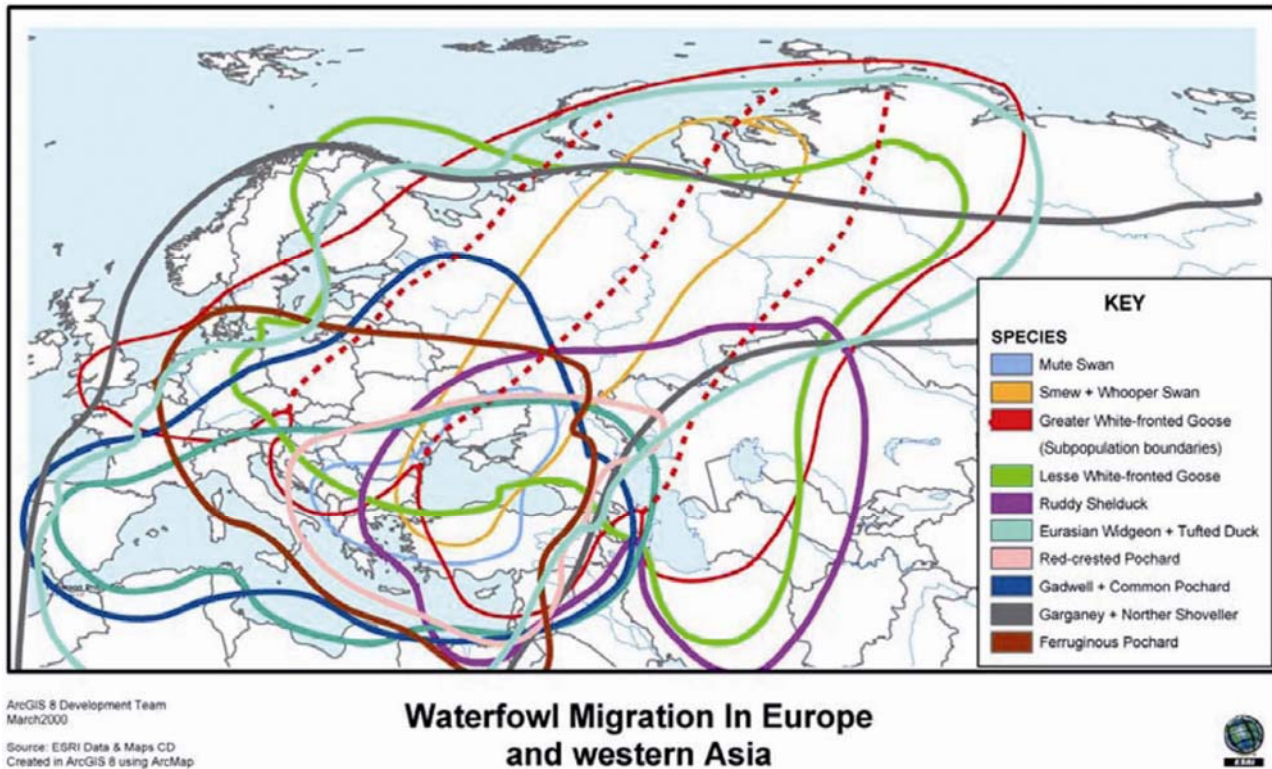
Table 2. HPAI detection in Europe (Brown, 2006).

Country (estimated 1st report)	Wild birds	Poultry
Turkey (Oct 2005)	+	+
Romania (Oct 2005)	+	+
Croatia (Oct 2005)	+	-
Ukraine (Dec 2005)	?	+
Azerbaijan (Jan 2006)	+	+
Bulgaria (Jan 2006)	+	-
Greece (Jan 2006)	+	-
Italy, Austria, Slovenia, Bosnia- H, Hungary, Poland, Slovakia, Sweden, Switzerland, Denmark, Albania, Serbia-Montenegro, Slovenia, UK (to April 2006)	+	-/+
France, Germany	+	+

They are distinguishable from the apparently chicken-adapted strains infecting domestic poultry in Turkey. This situation is also supported by wild bird immigration routes (SABIROVIC et al., 2006). The reports of the virus finding in dead migratory waterbirds raised concerns on the potential role of

these birds in the rapid dissemination of the virus over large geographic distances. Migratory waterbirds have been thought of a potential for the introduction of HPAI H5N1 virus to the countries (Figure 1).

Figure 1. Waterfowl immigration routes (Sabirovic et al., 2006).



Material and Method

Viruses. Total 173 virus isolates were used in this study (Table 3). The virus isolates analyzed in this study were isolated from domestic backyard and wild birds from the infected premises in Turkey. Viruses were detected by virus isolation in embryonating chicken eggs (ECEs). The isolates were typed as HPAI H5N1 viruses by hemagglutinin inhibition (HI) and neuraminidase inhibition tests with a panel of reference antisera (OIE, 2008). Viruses were received by the Veterinary Control and Research Institutes in Turkey (Bornova / İzmir, Pendik / İstanbul and Etlik Central / Ankara) in the form of tissue or allantoic fluid from 9-10 day-old SPF embryonated chicken eggs or RNA extract.

RNA Extraction and Reverse Transcriptase Polymerase Chain Reaction: RNA was extracted

using QIAamp RNA extraction kits (QIAGEN, Germany) according to the manufacturer's instruction. Primers were selected to recognise regions of hemagglutinin gene of the Avian Influenza A Virus as described by WHO (www.who.int/csr/diseases/ai). Amplification was performed with the forward primer H5F (5'- GCC ATT CCA CAA CAT ACA CCC -3') and reverse primer H5R (5'- CTC CCC TGC TCA TTG CTA TG -3'). All reactions were performed using SuperScript III One-Step RT-PCR System with Platinum *Taq* DNA according to the manufacturer's instruction (Invitrogen, USA). The PCR amplicons were visualised by Ethidium bromide staining after electrophoresis through 2% agarose gel (Sigma-Aldrich, Germany). The PCR products were separated in an agarose gel by electrophoresis and purified using QIAquick gel extraction kits (QIAGEN, Germany).

Table 3. HPAI (H5N1) viruses used in this study and GenBank accession numbers.

Sequence ID	Specific Host	Collection Date	GenBank Accession Number
HPAI (H5N1) viruses in Turkey in 2006			
A/WildDuck/Turkey/06/Beyazari/33/2006	WildDuck	2006	EU542733
A/Chicken/Turkey/56/Pervari/684/2006	Chicken	2006	EU542734
A/Chicken/Turkey/56/Merkez/683/2006	Chicken	2006	EU542735
A/Chicken/Turkey/56/Kurtalan/1458/2006	Chicken	2006	EU542736
A/Chicken/Turkey/06/Bala/1675/2006	Chicken	2006	EU542737
A/Chicken/TR/06/cubuk/1680/2006	Chicken	2006	EU542738
A/Turkey/TR/37/Merkez/1696/2006	Turkey	2006	EU542739
A/WildDuck/TR/37/Abana/1703/2006	WildDuck	2006	EU542740
A/Chicken/TR/36/Merkez/1542/2006	Chicken	2006	EU542741
A/Chicken/TR/55/Terme/17/2006	Chicken	2006	EU542742
A/Chicken/TR/60/Niksar/26/2006	Chicken	2006	EU542743
A/Duck/TR/61/Merkez/36/2006	Duck	2006	EU542744
A/Chicken/TR/55/Bafra47/2006	Chicken	2006	EU542745
A/Owl/TR/60/Niksar/187/2006	Owl	2006	EU542746
A/WildSwan/TR/74/Amasra/2005/2006	WildSwan	2006	EU542747
A/Pigeon/TR/21/Merkez/1334/2006	Pigeon	2006	EU542748
A/Turkey/TR/21/Merkez/1340/2006	Turkey	2006	EU542749
A/Chicken/TR/21/Merkez/1338/2006	Chicken	2006	EU542750
A/Turkey/TR/36/Merkez/1598/2006	Turkey	2006	EU542751
A/Sparrow/TR/55/Bafra/1095/2006	Sparrow	2006	EU542752
A/Chicken/TR/25/Merkez/1584/2006	Chicken	2006	EU542753
A/Chicken/TR/57/Boyabat/1081/2006	Chicken	2006	EU542754
A/Chicken/TR/55/Vezirkopru/1082/2006	Chicken	2006	EU542755
A/Chicken/TR/53/Cayeli/1085/2006	Chicken	2006	EU542756
A/WildSwan/TR/55/Merkez/929/2006	WildSwan	2006	EU542757
A/Turkey/TR/72/Merkez/1282/2006	Turkey	2006	EU542758
A/Duck/TR/21/Silvan/1201/2006	Duck	2006	EU542759
A/Turkey/TR/21/Silvan/1209/2006	Turkey	2006	EU542760
A/Chicken/TR/21/Silvan/1212/2006	Chicken	2006	EU542761
A/Duck/TR/21/Bismil/1219/2006	Duck	2006	EU542762
A/Chicken/TR/73/Silopi/1329/2006	Chicken	2006	EU542763
A/Chicken/TR/23/Merkez/1331/2006	Chicken	2006	EU542764
A/Chicken/TR/62/Cemisgezek/1336/2006	Chicken	2006	EU542765
A/Chicken/TR/21/Cermik/996/2006	Chicken	2006	EU542766
A/Chicken/TR/55/Merkez/10/2006	Chicken	2006	EU542767
A/Duck/TR/61/Merkez/35/2006	Duck	2006	EU542768
A/WildBird/TR/55/Bafra/29/2006	WildBird	2006	EU542769
A/Chicken/TR/60/Yesilyurt/939/2006	Chicken	2006	EU542770

A/Chicken/TR/55/Tekkekoy/1014/2006	Chicken	2006	EU542771
A/Chicken/TR/55/Tekkekoy/1068/2006	Chicken	2006	EU542772
A/Chicken/TR/55/19Mayis/1072/2006	Chicken	2006	EU542773
A/Goose/TR/66/Akdagmadeni/1543/2006	Goose	2006	EU542774
A/Goose/TR/36/Selim/1547/2006	Goose	2006	EU542775
A/Chicken/TR/55/Atakum/1083/2006	Chicken	2006	EU542776
A/Chicken/TR/55/Carsamba/1076/2006	Chicken	2006	EU542777
A/Chicken/TR/55/Terme/1075/2006	Chicken	2006	EU542778
A/Chicken/TR/55/Terme/1041/2006	Chicken	2006	EU542779
A/Chicken/TR/55/Bafra/1040/2006	Chicken	2006	EU542780
A/Chicken/TR/55/Carsamba/1037/2006	Chicken	2006	EU542781
A/Sparrow/TR/55/Bafra/1096/2006	Sparrow	2006	EU542782
A/Chicken/TR/55/Tekkekoy/1015/2006	Chicken	2006	EU542783
A/Chicken/TR/05/Merkez/1058/2006	Chicken	2006	EU542784
A/Chicken/TR/55/Bafra/1062/2006	Chicken	2006	EU542785
A/Chicken/TR/55/Terme/1065/2006	Chicken	2006	EU542786
A/Chicken/TR/55/Merkez/1067/2006	Chicken	2006	EU542787
A/Chicken/TR/55/Terme/1069/2006	Chicken	2006	EU542788
A/Chicken/TR/55/Terme/1071/2006	Chicken	2006	EU542789
A/Chicken/TR/55/Terme/1070/2006	Chicken	2006	EU542790
A/Chicken/TR/55/Terme/1073/2006	Chicken	2006	EU542791
A/Chicken/TR/55/Tekkekoy/1074/2006	Chicken	2006	EU542792
A/Chicken/TR/53/Merkez/1078/2006	Chicken	2006	EU542793
A/Chicken/TR/55/carsamba/1080/2006	Chicken	2006	EU542794
A/Chicken/TR/55/Asarcik/1084/2006	Chicken	2006	EU542795
A/Chicken/TR/57/Turkeli/1086/2006	Chicken	2006	EU542796
A/Chicken/TR/55/Tekkekoy/1087/2006	Chicken	2006	EU542797
A/Pigeon/TR/05/Merkez/1090/2006	Pigeon	2006	EU542798
A/Chicken/TR/55/Bafra/1091/2006	Chicken	2006	EU542799
A/Chicken/TR/57/Merkez/1092/2006	Chicken	2006	EU542800
A/Chicken/TR/60/Zile/1093/2006	Chicken	2006	EU542801
A/Duck/TR/60/Yesilyurt/1094/2006	Duck	2006	EU542802
A/Chicken/TR/55/Alacam/1097/2006	Chicken	2006	EU542803
A/WildBird/TR/55/Bafra/ist1/2006	WildBird	2006	EU542804
A/Pigeon/TR/55/Samsun/ist2/2006	Pigeon	2006	EU542805
A/Chicken/TR/58/Sarkisla/ist3/2006	Chicken	2006	EU542806
A/Chicken/TR/53/Limankoy/ist4/2006	Chicken	2006	EU542807
A/Chicken/TR/57/Lalakoy/ist5/2006	Chicken	2006	EU542808
A/WildBird/TR/52/Unye/ist6/2006	WildBird	2006	EU542809
A/Chicken/TR/57/Kabali/ist7/2006	Chicken	2006	EU542810
A/Chicken/TR/60/Niksar/ist8/2006	Chicken	2006	EU542811

A/Chicken/TR/55/Bafra/ist9/2006	Chicken	2006	EU542812
A/Chicken/TR/60/Tokat/ist10/2006	Chicken	2006	EU542813
A/Chicken/TR/52/Aydintepe/ist11/2006	Chicken	2006	EU542814
A/Chicken/TR/55/Yaylacati/ist12/2006	Chicken	2006	EU542815
A/Chicken/TR/60/Cikrik/ist13/2006	Chicken	2006	EU542816
A/Chicken/TR/55/Taflan/ist15/2006	Chicken	2006	EU542817
A/WildBird/TR/55/Samsun/ist16/2006	WildBird	2006	EU542818
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A/Chicken/TR/53/Baskoy/ist21/2006	Chicken	2006	EU542823
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A/Chicken/TR/55/Koruluk/ist25/2006	Chicken	2006	EU542827
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A/WildBird/TR/55/Kurupelit/ist27/2006	WildBird	2006	EU542829
A/Chicken/TR/57/Cakildak/ist28/2006	Chicken	2006	EU542830
A/HouseSparrow/TR/55/Ladik/ist29/2006	HouseSparrow	2006	EU542831
A/Chicken/TR/55/Camalan/ist30/2006	Chicken	2006	EU542832
A/Chicken/TR/53/Pazar/ist31/2006	Chicken	2006	EU542833
A/Chicken/TR/57/Doguca/ist32/2006	Chicken	2006	EU542834
A/WildBird/TR/55/Engiz/ist33/2006	WildBird	2006	EU542835
A/Chicken/TR/55/Kuscular/ist34/2006	Chicken	2006	EU542836
A/Chicken/TR/53/Komurculer/ist35/2006	Chicken	2006	EU542837
A/Chicken/TR/53/Ocak/ist36/2006	Chicken	2006	EU542838
A/Chicken/TR/55/Ucpinar/ist37/2006	Chicken	2006	EU542839
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A/WildBird/TR/55/Carsamba/ist39/2006	WildBird	2006	EU542841
A/WildBird/TR/55/Bafra/ist40/2006	WildBird	2006	EU542842
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A/Chicken/TR/56/Siirt/Bor399/2006	Chicken	2006	EU542859
A/Chicken/TR/15/Burdur/Bor453-1/2006	Chicken	2006	EU542860
A/Chicken/TR/49/Bulanik/Bor463/2006	Chicken	2006	EU542861
A/Chicken/TR/72/Batman/Bor543/2006	Chicken	2006	EU542862
A/Chicken/TR/21/Diyarbakir/Bor544/2006	Chicken	2006	EU542863
A/Chicken/TR/72/Kozluk/Bor545/2006	Chicken	2006	EU542864
A/Chicken/TR/72/Kozluk/Bor546/2006	Chicken	2006	EU542865
A/Quail/TR/72/Batman/Bor547/2006	Quail	2006	EU542866
A/Chicken/TR/21/Diyarbakir/Bor548/2006	Chicken	2006	EU542867
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A/Chicken/TR/21/Ergani/Bor551/2006	Chicken	2006	EU542870
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A/Chicken/TR/63/Viransehir/Bor577-1/2006	Chicken	2006	EU542873
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A/Chicken/TR/44/Malatya/Bor655/2006	Chicken	2006	EU542879
A/Chicken/TR/44/Malatya/Bor656/2006	Chicken	2006	EU542880
HPAI (H5N1) viruses in Turkey in 2007			
A/Chicken/Turkey/72/Gercus/329/2007	Chicken	2007	EU542881
A/Chicken/Turkey/21/Silvan/386/2007	Chicken	2007	EU542882
A/Chicken/Turkey/72/Merkez/367/2007	Chicken	2007	EU542883
A/Chicken/Turkey/72/Merkez/439/2007	Chicken	2007	EU542884
A/Goose/Turkey/21/Bismil/446/2007	Goose	2007	EU542885
A/Chicken/Turkey/21/Bismil/447/2007	Chicken	2007	EU542886
A/Chicken/Turkey/72/Merkez/449/2007	Chicken	2007	EU542887
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A/Chicken/Turkey/21/Silvan/455/2007	Chicken	2007	EU542892

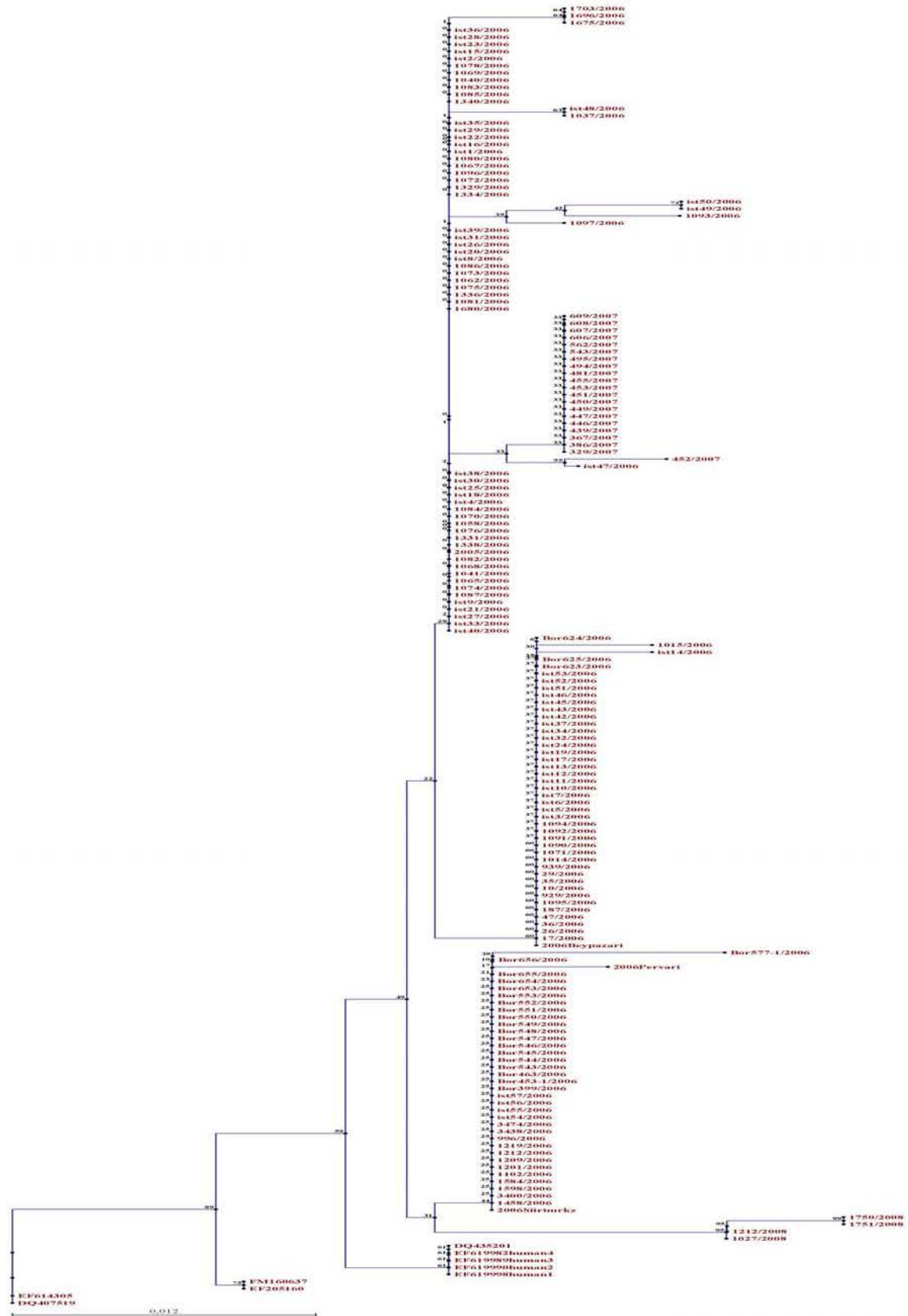
A/Chicken/Turkey/72/Merkez/481/2007	Chicken	2007	EU542893
A/Chicken/Turkey/72/Merkez/494/2007	Chicken	2007	EU542894
A/Sparrow/Turkey/72/Merkez/495/2007	Sparrow	2007	EU542895
A/Chicken/Turkey/21/Silvan/543/2007	Chicken	2007	EU542896
A/Chicken/Turkey/72/Merkez/562/2007	Chicken	2007	EU542897
A/Turkey/Turkey/72/Kozluk/606/2007	Turkey	2007	EU542898
A/Chicken/Turkey/72/Kozluk/607/2007	Chicken	2007	EU542899
A/Chicken/Turkey/72/Besiri/608/2007	Chicken	2007	EU542900
A/Turkey/Turkey/72/Kozluk/609/2007	Turkey	2007	EU542901
HPAI (H5N1) viruses in Turkey in 2008			
A/Chicken/Turkey/67/Caycuma/1027/2008	Chicken	2008	EU542902
A/Chicken/Turkey/67/Caycuma/1212/2008	Chicken	2008	EU542903
A/Chicken/Turkey/55/Samsun/1750/2008	Chicken	2008	EU542904
A/Chicken/Turkey/55/Samsun/1751/2008	Chicken	2008	EU542905

Sequence Analysis: The products of positive PCR amplification were sequenced by cyclesequencing reactions using ABI BigDye® Terminator v3.1 Cycle Sequencing Kit chemistry with template, H5F (3.2 pmoles) or primer H5R (3.2 pmoles) and BigDye® Ready Reaction Mix according to the manufacturer's instruction. Sequenced products were cleaned with a DyeEx 2.0 Nucleospin nucleotide removal kit (QIAGEN, Germany). Automated fluorescence sequencing was performed with an ABI PRISM® 310 Genetic Analyzer (Applied Biosystems, USA).

Phylogenetic Analysis: The sequences were edited and aligned using CLC COMBINEDWB3

software (CLC, 2007). Further sequences of HPAI H5N1 isolate from Turkey and neighbouring countries were obtained from GenBank (Table 4). Additional Asian-like H5N1 sequences, used for comparison, were obtained from the National Centre for Biotechnology Information (NCBI) (Table 4). The phylogenetic tree (Figure 2) was generated using CLC COMBINEDWB3 software. Phylogenetical analysis was done by using HA partial gene sequences. Phylogenetical tree was built by Neighbor-Joining method; matrix of distances was counted with p-distance algorithm. Reliability of clades was checked with bootstrap analysis with 100 replications.

Figure 2. Phylogeny of HA gene of HPAI (H5N1).



Nucleotide sequence accession numbers. The sequences reported in this paper have been deposited in the GenBank database under accession numbers indicated that table 3. The other sequences that used for comparison were reached from GenBank and these indicated table 4.

Table 4. Additional sequences used for comparison.

Accession Numbers	References
DQ407519	Brown and others, 2006
EF619998	Lin, 2007
EF619990	Lin, 2007
EF619989	Lin, 2007
EF619982	Lin, 2007
EF205160	Lipatov and others 2007
FM160637	Owoade and others, 2008
EF614305	Turcitu and others, 2006
DQ435201	Yingst and others, 2006

Findings

All viruses were diagnosed as Highly Pathogenic Avian Influenza A (H5N1) viruses by RT-PCR. Partial-HA gene characterisation of all isolates revealed that the Turkish H5N1 viruses were highly similar to Asian and European H5N1 isolates in all genes from the same time period (Figure 2). The HA cleavage site (PQGERRRKKRGLF and PQGERRRKKRGLF) identified the all H5N1 viruses isolated between 2005 and 2008 (Table 5). WHO recommended primer set were used and this primer set has given us partial Haemagglutinin (HA) gene sequencing (220 bp) of isolates. The Kızıksa Village/Manyas isolate (DQ507519) was used as a referens outgroup for phylogeny (Figure 2). Also sequence of this isolate was same with Romanian isolate (EF614305). 2006, 2007 and 2008 isolates show close relationship with eachother and other European and Asian isolates (Figure 2).

Table 5. Protein structure of HA cleavage site

Years of isolates	Protein structure of HA cleavage site
2005	PQGERRRKKRGLF
2006	PQGERRRKKRGLF
2007	PQEERRRKKRGLF
2008	PQGERRRKKRGLF

Discussion and Conclusion

The present study provides molecular properties of the Turkish HPAI isolates. The information in the present study will be valuable for understanding HPAI isolates from different countries affected by the HPAI outbreak in around Turkey.

Genetic analysis of the all isolates shows that Turkish, European and Asian HPAI H5N1 viruses were highly similar (Figure 2). There was a level of relationship between different outbreaks in Europe, Turkey and Asia. Varying host specificity can be discussed. Turkish HPAI H5N1 virus isolates are almost identical to the virus isolated in wild birds in Central Asia during this time. These data were also confirmed by VLA Weybridge. The European isolates of the Asian HPAI H5N1 type of virus could be classified in closely related clades (BROWN et al., 2006). These clades include virus isolates that have been obtained from various village poultry in Turkey and have clearly been circulating in direct poultry-poultry infections (SABIROVIC et al., 2006). Results suggested that there were direct relation between the affected areas in China (Qinghai lake), in Russia, in southern Siberia (Novosibirsk), in the Europe, in Iraq and in Africa (Figure 2).

This study includes only partial Haemagglutinin (HA) gene sequencing (220 bp) of Turkish isolates. But country needs full HA partial gene sequencing result of the some indicative isolates. This is necassary to prepare epidemiological analyses and to conflict of disease. There are only few isolates that sequenced full HA gene by VLA Weybridge (BROWN et al., 2006) and WHO Collaborating Centre for Reference and Research on Influenza (LIN, 2007). It is important to collect standard core set of data from all outbreaks. Continuing HPAI H5N1 outbreaks in Asia, Europe and the Middle East emphasize the importance of full genetic characterizations of different viruses from different countries and of pathogenesis studies using different animal species.

The questions that remain regarding the Turkish HPAI H5N1 2006 outbreak are where the virus originated and how this virus spread to at least all country. It is possible that wild birds may have played a role in introducing the viruses into different regions of country in 2006. The wide

prevalence of HPAI H5N1 viruses throughout Turkey in 2006 suggests that viruses may have spread to different regions by the introduction of infected wild animals. But It is unknown at present whether HPAI H5N1 infection will persist in wild bird populations throughout the year in the absence of further introductions. For this reason, epidemiological studies are required in Turkey in the areas where infection has been found in wild birds to identify the domestic poultry flocks that could be regarded as at risk.

As a result, this study provides a characterization of recent HPAI H5N1 viruses isolated from Turkey that caused between 2005 and 2008, which has a relationship with HPAI H5N1 outbreak in neighboring countries. To avoid future outbreaks, we need a clear understanding of how this unprecedented epidemic began. Therefore, further characterization of the HPAI H5N1 viruses is urgently needed.

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