



# CASE REPORT: ISOLATION OF A LOW PATHOGENIC AVIAN INFLUENZA LPAI (H7N7) FROM A BLACK KITE (MILVUS MIGRANS) IN EGYPT IN 2004

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## SUMMARY

Avian influenza is endemic in some species of wild birds and is generally believed to cause only an asymptomatic infection. These viruses routinely transmit from this wild bird reservoir to poultry in many areas all over the world. Low pathogenic avian influenza (LPAI) was previously reported in Egypt from different types of wild birds. This report describes the isolation and genetic characterization of H7N7 LPAI virus from a black kite, the first reported from this species, during surveillance done on wild birds in 2004. The black kite (*Milvus migrans*) is a migratory bird that has breeding habitat in Europe and migrates in the winter south to North Africa and the Middle East and occasionally may be a permanent resident there. Eight samples were collected in South Sinai and tested by virus isolation in embryonating chicken eggs. One sample had positive HA activity after the second passage in SPF embryos. Virus identification and characterization were done and the isolate was confirmed as H7N7 LPAI. The sequence data showed that this isolate was most closely related to European H7 strains isolated from domestic and wild birds.

**Keywords:** Low pathogenic avian influenza (LPAI), surveillance in wild and migratory birds, black kite (*Milvus migrans*), subtype H7N7.

## Introduction

Wild birds are the natural and often silent reservoir for avian influenza viruses because the infection is usually asymptomatic. If infected wild birds come into contact with or contaminate an area populated by poultry the virus may be transmitted to the flock providing an opportunity for the virus to proliferate and possibly mutate. Viruses introduced in this manner may start out as low pathogenic strains and mutate into highly pathogenic strains (14). Influenza, once introduced into poultry, can become endemic within the poultry population. (12). Avian influenza virus, either low pathogenic (ex. H9N2) or highly pathogenic strains have the potential to be zoonotic. Although no human deaths have been associated with any LPAI virus (8), these viruses may still have pandemic potential. Some LPAI H5 and H7 virus strains have mutated to HPAI following circulation in domestic poultry flocks (8, 9). LPAI viruses have been isolated previously from wild birds (11, 6), and many surveys have demonstrated that healthy wild birds were asymptomatic reservoirs of AI viruses. (12) Low pathogenic avian influenza viruses of the H7N7 subtype have been isolated from wild birds previously and have also been associated with highly pathogenic outbreaks from outbreaks in Germany, A/gull/Germany/79 (H7N7), (1, 2, 3, 5) and recently in Netherlands in 2003 (8), and in 1998 in Ireland and North Ireland, and LPAI (H7N7) in Germany in 2001 (15, 4). This report describes the isolation and genetic characterization of H7N7 LPAI virus during surveillance done on wild birds in 2004.

## Material and Methods

Cloacal swabs were collected during surveillance done on wild birds in South Sinai in 2004, where 8 samples were collected from apparently healthy wild black kites. The 8 samples were inoculated in 9-day-old specific pathogen free (SPF) embryonating chicken eggs for 7 days at 37°C. The allantoic fluids were harvested after both the first and second passages in SPF eggs and tested for HA activity as previously described (16). Agar gel Immunodiffusion test (AGID) was used for detection of common Matrix (M) protein and Nucleoprotein (NP) of avian influenza and for Newcastle disease virus (NDV) isolates by using reference antisera from (IZSVe, Padova, Italy) according to methods previously described (16). The RNA was extracted from the harvested allantoic fluids by using (QIAGEN, Valencia, Calif, USA) for use with different molecular diagnostic tests. The isolate was tested by RT-PCR for the M gene of AI by using primers and RT-PCR conditions as described in the WHO Manual (16). The sample was also tested using a commercial RRT-PCR (ADIA-VET; France) for the M gene as described by the kit manual. The isolated virus was also tested by using a commercial RT-PCR H5N1 kits (Roche, Mannheim, Germany) for subtyping of the H5 and N1 genes. The virus isolate was sub-typed by the hemagglutination inhibition (HI) test (10). Isolates of avian influenza were confirmed for detection of H7 gene of AI by (Sacace kit, Italy). Nucleotide sequence analysis of the HA gene was conducted to identify the pathogenicity of the isolated strains. The PCR products of the HA gene fragments were directly sequenced. Complete nucleotide sequencing of the HA gene was carried out in (NLQP), Egypt, and also confirmed in OIE Reference Laboratory, Veterinary Laboratories Agency, Weybridge, Surrey, UK. For the sequencing of isolate, Full-length amplification of HA gene. The amplified HA products were purified using a PCR purification kit (Qiagen, Hilden, Germany) and sequenced by using internal-specific primers to generate overlapping HA fragments using BigDye Terminator v3.1 Cycle Sequencing Kit on an automatic sequencer (ABI-3130; Applied Biosystems). Phylogenetic analysis was carried out on the full length HA gene. Multiple and pairwise sequence alignments were constructed using the ClustalV algorithm and phylogenetic tree were constructed using the neighbour-joining of MegAlign program from LaserGene Biocomputing Software Package (DNASTAR, Madison, WI).

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## Result

The 8 black kite samples were tested by viral isolation in SPF chicken embryos, a single sample had HA activity in the allantoic fluid after the second passage. The HA titer of the isolated virus was 7 log<sub>2</sub>. The sample was tested positive by the AGID test for avian influenza and was negative for NDV. The sample was positive by HI test with the H7 antisera and was negative for the other 14 subtypes. The isolate tested positive using RRT-PCR (ADIA-VET; France) for the M gene. The isolate also tested positive for detection of subtype H7 and negative for H5 by (Sacace kit, Italy) and negative by H5N1 RRT-PCR (Roche) kits. The neuraminidase (NA) subtype was determined at the VLA, Weybridge, Surrey, UK as N7 subtype. The full coding sequence of the HA gene was amplified and sequenced. The HA cleavage site sequence (PEIPKGRGLF) of the H7N7 virus included only 2 basic amino acids with no insertions that is consistent with LPAIV. The sequenced HA gene was submitted into Genbank (Accession number: FJ839886). Phylogenetic analysis of the HA gene of Egyptian isolate A/black kite/Egypt/054/2004(H7N7) showed the virus most closely grouped with European viruses from England and Germany and they were genetically distinct from more recent European isolates from Italy and the Netherlands as well as some Asian countries in the last decade. This isolate was completely separated from the North American strains. (Fig.1)

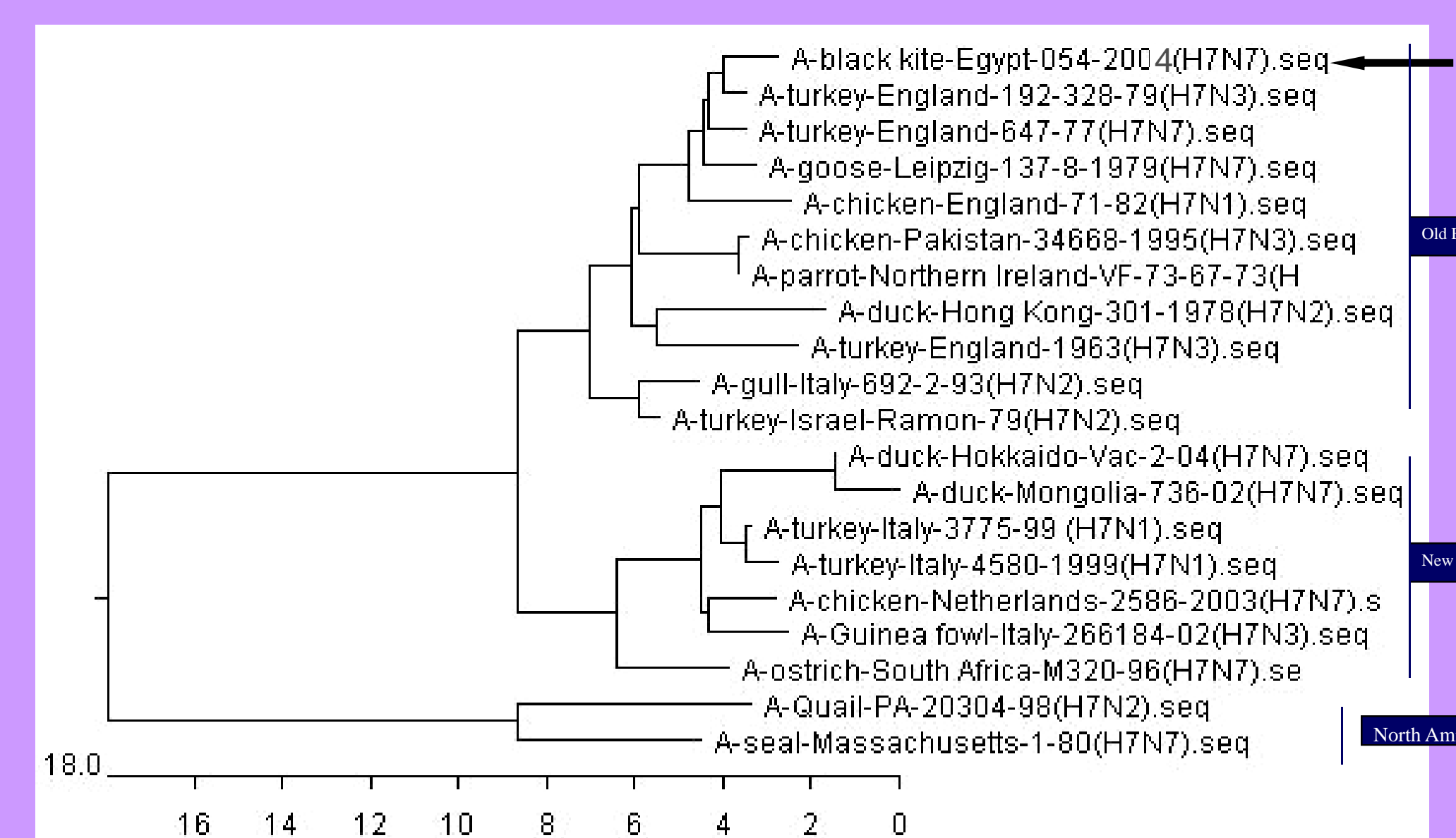


FIG. 1. PHYLOGENETIC ANALYSIS OF H7 GENE OF EGYPTIAN ISOLATE (A/BLACK KITE/2004) COMPARED TO OTHER H7 STRAINS. RESULTS ILLUSTRATE THE EGYPTIAN STRAIN IS CLUSTERED WITH OLD EURASIAN STRAINS. THE ANALYSES WERE DONE BY APPLYING THE CLUSTAL V, MEGALIGN PROGRAM (DNA STAR, MADISON, WI).

## Discussion

This report describes the isolation and genetic characterization of H7N7 LPAI from black kites (*Milvus migrans*) collected from wild birds in the South Sinai. Although an extensive number of AIV isolations have previously been reported from wild birds, the reservoir species and global distribution for many of these AIV subtypes are not completely understood (13). Sequence of the Egyptian isolate was compared with sequences from H7 AIVs isolated from commercial poultry and aquatic birds from Europe, Asia, and the Middle East, and the virus was most closely associated with several European isolates. Although avian influenza can likely infect any wild bird species, certain wild birds are considered the primary reservoir, including some ducks, gulls, and some shorebird species. These species usually have asymptomatic infections, and they may excrete virus in the feces for long periods and can even be infected with more than one subtype of avian influenza at one time. The virus even in wild birds appears to be seasonal, with mallard, pintail, and certain other duck species having the highest incidence of infection in the Fall of the year. Once a virus has transmitted to densely reared poultry, the virus has an opportunity to replicate quickly and has a potential to mutate to a more virulent form. The data from this work revealed that LPAI viruses of H7 is present in wild and migratory birds in Egypt and can be a cause for concern to spread to domestic poultry. The potential also exists for viral genes from these low pathogenic viruses to reassort with viruses already found in Egyptian poultry, including highly pathogenic H5N1, with the potential creation of viruses with new antigenic or biological features. Therefore monitoring and surveillance of the free living, wild and migratory birds for AI viruses in Egypt should remain a research priority as part of an international surveillance program.

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