**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 SPF 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 H7N7 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 asymptomatic area, 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 to highly pathogenic strains (14). Once introduced into poultry, can become endemic within the poultry population. (12). Avian influenza virus, either low pathogenic (e.g. 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 H7 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 (H7N1), (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 LPAI (H7N7) virus during surveillance done on wild birds in 2004.

**Materials and Methods**

Clausal swabs were collected during surveillance done on wild birds in South Sinai, Egypt in 2004, when 8 samples were collected from apparently healthy wild black kites. The 8 samples were inoculated in 8-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) (5). The results were confirmed by sequencing (GVS, Padova, Italy) according to methods previously described (16).

The RNA was extracted from the harvested allantoic fluids by using TRIZOL® (Life Technologies, CA, 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 described by Addlestone, C.J, Thomas P.J. An outbreak of highly pathogenic avian influenza virus in Egypt, 2004 (14). The sample was also tested using a commercial RT-PCR (AVI-DEA; France) for the H5 gene as described by the kit manual. The isolated virus was confirmed as RT-PCR H7N7 (Kito, Mannheim, Germany) for subtyping of the H5 and N1 genes. The virus isolate was subtyped by the hemagglutination inhibition (HI) assay using_hybrid typing of isolate and their valuable cooperation. The sequence data showed that this isolate was most closely related to European H7N7 strains isolated from domestic and wild birds.

**References**

7. Elbers, A.R., Fabi, T.H., de Vries, T.S., de Wil, J.J., Rippen, A. and Koch, G. The HPAI H7N7 virus isolated from an Old Eurasian 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 migrates in the winter south, and may be a permanent resident there. Eight samples were collected in South Sinai and tested by virus isolation in embryonating SPF 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 H7N7 strains isolated from domestic and wild birds.

**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 A/H7N7 isolates have been previously reported from wild birds, the seasonal cycle and global distribution for many of these A/H7N7 isolates is not clear. The H7N7 isolates from different countries described here were all characterized using similar molecular methodologies (13, 14) and may be from a related cluster of viruses. This work was kindly supported by Research grant from National Academy of Science, Egypt. 2003 – 2004.

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 log2. 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 antiserum and was negative for the other 14 subtypes. The isolate tested positive using RT-PCR (AVI-DEA; France) for the H7 gene. The isolate also tested positive for detection of subtype H7 and negative for H5 by (Saccate, kit, Italy) and negative by H5N1 RRT-PCR (Roche) kits. The neuraminidase (NA) gene subtype was determined at the VLA, Weybridge, Surrey, UK as subtype 8. The full coding sequence of the HA gene was amplified and sequenced (PEIPKRGFLF) of the H7N7 virus included only 2 basic amino acids with no insertions that is consistent with LPAI. The sequenced HA gene was submitted into Genbank (Accession number: F43584). 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 Europe and Germany and they were genetically distinct from many 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)