

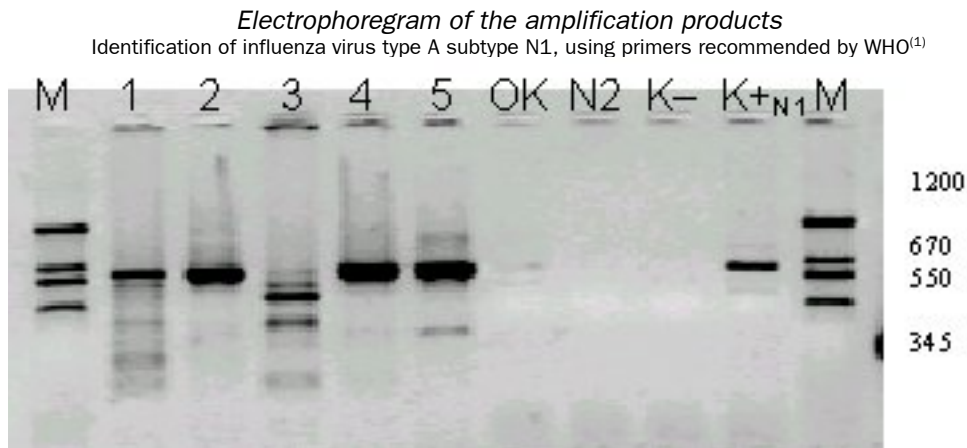
HIGHLY PATHOGENIC AVIAN INFLUENZA IN RUSSIA

Additional information

Translation of information received on 5 August 2005 from Dr Evgueny A. Nepoklonov, Head of the Main Veterinary Department, Ministry of Agriculture and Food, Moscow:

Report date: 5 August 2005.

Structure of the haemagglutinin and neuraminidase genes of influenza virus isolates from Novosibirsk region



M marker
OK negative control RNA extraction
N2 H5N2 strain cDNA
K- PCR⁽²⁾ negative control
K+N₁ PCR positive control (H1N1 strain cDNA)

Sample No. 1 – homogenate of internal organs (spleen, trachea, lungs, brain) from two domestic ducks from the private sector, Kopkul village, Kupino district, Novosibirsk region;

Sample No. 2 – homogenate of internal organs (spleen, trachea, lungs, brain) from a domestic duck from the private sector, Kopkul village, Kupino district, Novosibirsk region;

Sample No. 3 – homogenate of internal organs (spleen, trachea, lungs, brain) from a wild duck from Suzdalka village, Dovolnoye district, Novosibirsk region;

Sample No. 4 – homogenate of internal organs (spleen, trachea, lungs, brain) from a goose from the private sector from Suzdalka village, Dovolnoye district, Novosibirsk region;

Sample No. 5 – homogenate of internal organs (spleen, trachea, lungs, brain) from a chicken from the private sector from Suzdalka village, Dovolnoye district, Novosibirsk region.

According to the electrophoregram, the type A virus found in the samples of material is related to subtype H5N1.

For the confirmation of the PCR results, the amplified cDNA fragments (of two loci of the haemagglutinin gene and of the fragment of the neuraminidase gene) were sequenced. The results of the sequencing confirmed that the type A virus found in the samples of material is related to subtype H5N1.

In order to obtain additional information about the virulence of the isolates (by analysing the structure of the haemagglutinin cleavage site), these parts were amplified and the fragments of PCR sequenced. That research showed that the virus sequence is **PQGERRRKKR/GL**, which is analagous to the sequence of the highly virulent virus found in three species of migratory water birds during the epidemic in May/June on Qinghai Lake (People's Republic of China)⁽³⁾.

The nucleotid sequences of the PCR fragments of two other parts of haemagglutinin and neuraminidase also showed the highest similarity with the nucleotid sequences of the isolates from infected mountain geese and gulls at the time of that epidemic.

Sequence data at the cleavage site for an isolate from a turkey
from the village of Suzdalka, Dovolnoye district, Novosibirsk region
(Information provided by Vector, the State Scientific Centre for Virology and Biotechnology)

A-Turkey-suz H5N1 vs HCO

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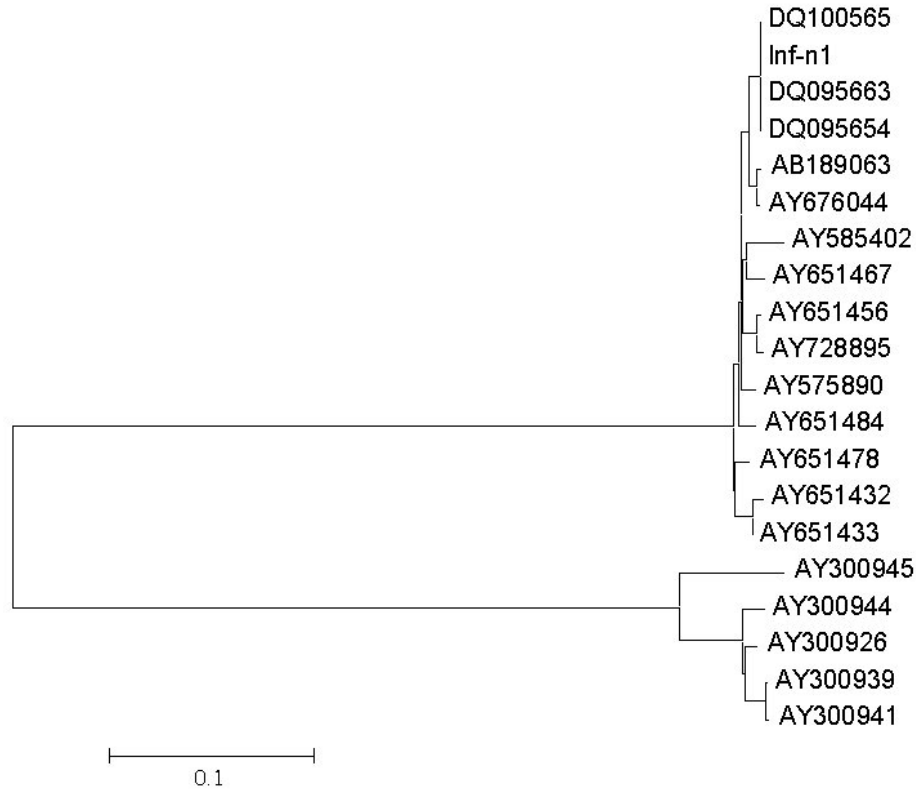
                *           20           *           40           *           60
AY651368 : PKYVKSSRLVLVLATGLRNSPQRERRPKRRRGLFGAIAAGFIEGGWQGMVDGWYGYHHSNEQGSGY : 62
A-Tu-suz  : PKYVKSSRLVLVLATGLRNSPQGERRRRPKRRRGLFGAIAAGFIEGGWQGMVDGWYGYHHSNEQGSGY : 62

                *           80           *           100          *           120
AY651368 : AADKESTQKAIDGVTNKVNSILDKMNTQFEAVGREFNNLERRIENLNKKMEDGFLDVWTYNA : 124
A-Tu-suz  : AADKESTQKAIDGVTNKVNSILDKMNTQFEAVGREFNNLERRIENLNQKMEDGFLDVWTYYA : 124
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LOCUS AY651368 1697 bp RNA linear VRL 16-JUL-2004
DEFINITION Influenza A virus (A/Ck/ST/4231/2003(H5N1)) hemagglutinin (HA) gene, partial cds.
ACCESSION AY651368
VERSION AY651368.1 GI:50296120
KEYWORDS .
SOURCE Influenza A virus (A/Ck/ST/4231/2003(H5N1))
ORGANISM Influenza A virus (A/Ck/ST/4231/2003(H5N1))
Viruses; ssRNA negative-strand viruses; Orthomyxoviridae; Influenzavirus A.
REFERENCE 1 (bases 1 to 1697)
AUTHORS Li,K.S., Guan,Y., Wang,J., Smith,G.J., Xu,K.M., Duan,L., Rahardjo,A.P., Puthavathana,P., Buranathai,C., Nguyen,T.D., Estoepangestie,A.T., Chaisingh,A., Auewarakul,P., Long,H.T., Hanh,N.T., Webby,R.J., Poon,L.L., Chen,H., Shortridge,K.F., Yuen,K.Y., Webster,R.G. and Peiris,J.S.
TITLE Genesis of a highly pathogenic and potentially pandemic H5N1 influenza virus in eastern Asia
JOURNAL Nature 430 (6996), 209-213 (2004)
PUBMED 15241415
REFERENCE 2 (bases 1 to 1697)
AUTHORS Li,K.S., Guan,Y., Wang,J., Smith,G.J.D., Xu,K.M., Duan,L., Rahardjo,A.P., Puthavathana,P., Buranathai,C., Nguyen,T.D., Estoepangestie,A.T.S., Chaisingh,A., Auewarakul,P., Long,H.T., Hanh,N.T.H., Lim,W., Webby,R.J., Poon,L.L., Chen,H., Shortridge,K.F., Yuen,K.Y., Webster,R.G. and Peiris,J.S.M.
TITLE Direct Submission
JOURNAL Submitted (14-JUN-2004) Microbiology, The University of Hong Kong, HKU Pathology Building, QueenMary Hospital, Pokfulam, Hong Kong SAR, China

According to the sequence data, the cleavage site structure is practically identical to that of the highly pathogenic strain of subtype H5N1 isolated in Hong Kong in 1997.

Phylogenetic tree constructed from fragment 640 of the nucleotids of the neuraminidase gene (referred to as *Inf-n1*) of the isolate (H5N1) from a turkey from Suzdalka, Novosibirsk region (Information provided by Vector, the State Scientific Centre for Virology and Biotechnology)



Explanation of codes in the chart according to GenBank data (the closest isolates are in bold):

AY300926	A/chukkar/MN/14591-7/98(H5N2)
AY300939	A/duck/NJ/117228-7/01(H5N2)
AY300941	A/duck/ME/151895-7A/02(H5N2)
AY300944	A/duck/NY/191255-79/02(H5N2)
AY300945	A/turkey/CA/D0208652-C/02(H5N2)
AY575890	A/Ck/HK/96.1/02 (H5N1)
AY585402	A/duck/Fujian/19/2000(H5N1)
AY651432	A/Ck/Indonesia/BL/2003(H5N1)
AY651433	A/Ck/Indonesia/PA/2003(H5N1)
AY651456	A/Dk/Viet Nam/11/2004(H5N1)
AY651467	A/Ck/HK/NT93/2003(H5N1)
AY651478	A/Ph/ST/44/2004(H5N1)
AY651484	A/Dk/YN/6445/2003(H5N1)
AY676044	A/duck/Korea/ESD1/03(H5N1)
AY728895	A/chicken/Viet Nam/HauGiang-617/2004(H5N1)
DQ095654	A/Great Black-headed Gull/Qinghai/2/05(H5N1)
DQ095663	A/Bar-headed Goose/Qinghai/67/05(H5N1)
AB189063	A/crow/Osaka/102/2004(H5N1)
DQ100565	A/great black-headed gull/Qinghai/1/2005(H5N1)

According to the phylogenetic tree, the preliminary data on the neuraminidase structure show that it is practically identical to that of the isolate from bar-headed geese (*Anser indicus*) from the Qinghai Lake region (People's Republic of China).

(1) WHO: World Health Organization

(2) PCR: polymerase chain reaction

(3) J. Liu, H. Xiao, F. Lei. *et al.* Highly Pathogenic H5N1 Influenza Virus Infection in Migratory Birds. www.sciencexpress.org/ 6 July 2005 / Page 1/ 10.1126/science.1115273