HAL
open science

Field and laboratory findings of the first incursion of the Asian H5N1 highly pathogenic avian influenza virus in Africa

Paola de Benedictis, Tony Manuel Joannis, Lami Hannatu Lombin, Ismaila Shittu, Maria Serena Beato, Valeria Rebonato, Giovanni Cattoli, Ilaria Capua

To cite this version:

Paola de Benedictis, Tony Manuel Joannis, Lami Hannatu Lombin, Ismaila Shittu, Maria Serena Beato, et al.. Field and laboratory findings of the first incursion of the Asian H5N1 highly pathogenic avian influenza virus in Africa. Avian Pathology, 2007, 36 (02), pp.115-117. 10.1080/03079450601161406 . hal-00540069

HAL Id: hal-00540069

https://hal.science/hal-00540069v1

Submitted on 26 Nov 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Field and laboratory findings of the first incursion of the Asian H5N1 highly pathogenic avian influenza virus in Africa

Journal:	*Avian Pathology*
Manuscript ID:	CAVP-2006-0141.R1
Manuscript Type:	Short Communication
Date Submitted by the Author:	15-Oct-2006
Complete List of Authors:	De Benedictis, Paola; OIE FAO and National reference laboratory for newcastle disease and avian influenza, Istituto Zooprofilattico Sperimentale delle Venezie, Virology department Joannis, Tony; National Veterinary Research Institute, P.M.P. 01 VOM, Plateau State Lombin, Lami; National Veterinary Research Institute, P.M.P. 01 VOM, Plateau State Shittu, Ismaila; National Veterinary Research Institute, P.M.P. 01 VOM, Plateau State Beato, Maria Serena; OI/FAO and National reference laboratory for Avian Influenza and Newcastle disease, Virology Laboratory Rebonato, Valeria Cattoli, Giovanni; OIE/FAO and national reference laboratory for Avian Influenza and Newcastle disease, Virology laboratory Capua, Ilaria; OIE/FAO and national reference laboratory for Avian Influenza and Newcastle disease, Virology laboratory
Keywords:	H5N1 , HPAI, Africa, phylogeny

SCHOLARONE™
Manuscripts

For Peer Review Only

Cavp-2006-0141.R1

Field and laboratory findings of the first incursion of the Asian H5N1 highly pathogenic avian influenza virus in Africa

Paola De Benedictis[1], Tony Manuel Joannis[2], Lami Hannatu Lombin[2], Ismaila Shittu[2], Maria Serena Beato[1], Valeria Rebonato[1], Giovanni Cattoli[1], and Ilaria Capua[1*]

[1]OIE, FAO and National Reference Laboratory for Newcastle Disease and Avian Influenza, Istituto Zooprofilattico Sperimentale delle Venezie, Viale dell'Università 10, 35020, Legnaro, Padova, Italy [2]National Veterinary Research Institute, P.M.P. 01 VOM, Plateau State, Nigeria

Short Title: First incursion of Asian H5N1 in Africa

[*]Corresponding author: Ilaria Capua, e-mail: icapua@izsvenezie.it;

tel: +39.049.8084369, fax: +39.049.8084360

Keywords: H5N1, HPAI, Africa, phylogeny

Running title: H5N1 in Nigeria

Received: 14 September 2006

Formatted: Right
Formatted: Font: 14 pt
Formatted: Left
Formatted: Font: 14 pt
Formatted: Font: Bold
Formatted: Font: Bold
Formatted: Font: Italic
Formatted: Font: Italic, Italian (Italy)
Formatted: Left, None
Formatted: Italian (Italy)
Deleted: ¶
Formatted: Font: Italic, Italian (Italy)
Deleted: ¶
Formatted: Font: Italic, Italian (Italy)
Formatted: Font: Italic, English (U.S.)
Formatted: Left
Formatted: English (U.S.)
Formatted: Left
Formatted: Font: Italic

For Peer Review Only

Cavp-2006-0141.R1

Field and laboratory findings of the first incursion of the Asian H5N1 highly pathogenic avian influenza virus in Africa

Paola De Benedictis[1], Tony Manuel Joannis[2], Lami Hannatu Lombin[2], Ismaila Shittu[2], Maria Serena Beato[1], Valeria Rebonato[1], Giovanni Cattoli[1], and Ilaria Capua[1]

Formatted: Italian (Italy)

Formatted: Italian (Italy)

Abstract

In mid January 2006, an outbreak of avian influenza in domestic birds was recorded in Kaduna State, Nigeria. The virus responsible for the outbreak was characterised as a highly pathogenic avian influenza (HPAI) H5N1 (A/chicken/Nigeria/641/06), belonging to the Qinghai lineage. The index case occurred on a farm containing mixed avian species including free-range birds. Clinical signs and mortality were observed in chickens, geese and ostriches. The present paper describes the clinical, pathological and virological findings of this outbreak.

Deleted: in

Deleted: premise

For Peer Review Only

Introduction

The H5N1 virus poses a significant threat both to animal and human health as it has been shown to be lethal for poultry and able to infect mammals, including humans. Prior to the H5N1 epidemic, HPAI had only seldom affected the African continent. Only two outbreaks had been reported, one in wild terns (*Sterna hirundo*) in 1961 (Becker, 1967), and the other, caused by an H5N2 virus affected intensively reared ostriches in South Africa (Olivier, 2006). Both outbreaks were self–limiting.

In mid January 2006, the first suspicion of HPAI was reported in Kaduna State in Northern Nigeria. The outbreak affected several avian species on the same commercial farm.

In this paper we describe the clinical and pathological findings in the avian species involved in the African outbreak during the first months of 2006 and we also report the virological findings of the first isolates from the African continent.

Deleted: has

Deleted: To date, only

Deleted: have

Formatted: Indent: First line: 35.4 pt

Deleted: of

Material and Methods

Sick chickens exhibiting clinical signs and excess mortality were submitted for investigations to the National Veterinary Research Institute (NVRI), Vom, Nigeria. Following the suspicion of HPAI a field visit was arranged by NVRI. Pathological samples were shipped to the OIE/FAO Reference laboratory, IZSVe, Legnaro, Padua, Italy for attempted virus isolation. Samples of lungs, trachea, spleen, liver, and intestines were collected from chickens, geese and ostriches found dead or moribund and thereafter humanely killed. Samples were processed by Real-Time Reverse Transcriptase Polymerase Chain Reaction (RRT-PCR) targeting the M gene (Spackman *et al.*, 2002) and for virus isolation, subtyping and pathotyping as described by EC, 1992 and Alexander and Spackman, 1981). Sequences were generated directly using the Big Dye Terminator v3.1 cycle

Deleted: Ill

For Peer Review Only

sequencing kit (Applied Biosystems) in a 3100 Avant Genetic Analyser (Applied Biosystems), employing the primers listed in Table 1.

Results

The first outbreak involved about 47,000 birds of different species, mainly chickens, of different ages, reared bred on the same premises. A small number of geese, turkeys and ostriches, raised in the open, were also affected..

At the time of the field investigation all turkeys (37/37) and 2/4 geese had died. Of the remaining birds, 80% (37,000/46,000) of chickens and 4% (5/120) of adult ostriches had died.

The epidemiological inquiry was not substantiated by any documentation, although the NVRI team found verbal evidence that some of the chickens (commercial layers and breeders of different breeds and ages) had been purchased recently from different sources, most of which could not be traced.

The only clinical signs of the disease observed by NVRI veterinarians in ostriches were depression and sternal recumbency followed by death. All of the turkeys had died and been disposed of before the outbreak investigation started.

Typical HPAI clinical findings were seen in adult chickens, namely cyanotic combs and wattles, subcutaneous haemorrhages and oedema of the limbs. Nervous and respiratory signs were also observed.

Pathological examination in ostriches revealed paint-brush and diffuse haemorrhages in the epicardium and serosal surface of the jejunum and ileum, petechial haemorrhages on the serosal surface of the duodenum and severe haemorrhages of the ovarian follicles.

Adult chickens displayed diffuse haemorrhages in the respiratory and enteric tracts, the latter including the proventriculus. In some birds the spleen and ovarian follicles were congested and haemorrhagic. No data was available on the geese and turkeys, except for very high mortality.

Formatted: Indent: First line: 35.4 pt

Deleted: recently

Formatted: Indent: First line: 35.4 pt

Formatted: Indent: First line: 35.4 pt

For Peer Review Only

The first HPAI H5N1 isolate (A/ck/Nigeria/641/06) was fully sequenced (Genbank accession numbers: DQ406728, DQ52929–DQ529297 and DQ780008) and aligned with the sequenced genomes of other recent A/H5N1 viruses currently available in GenBank. Phylograms constructed from both nucleotide and amino acid sequences of all genes show a similar topology consisting of a major cluster, containing A/H5N1 derived from the Qinghai lake lineage (A/Bar-headed Goose/Qinghai/60/2005, H5N1), together with other recent Eurasian isolates. The HA tree in Figure 1 was selected as representative of the remaining seven genes phylograms. The Eurasian-African cluster is clearly distinguishable from the A/H5N1 lineages circulating in the Far East (homology ≤ 95.8%). The phylogenetic data indicates a high degree of homology (99.6%) with the lineage progenitor, including two mutations associated with virulence, namely Lysine residue in position 627 of the PB2 gene, which has been associated with an enhanced replication in the mammalian host (Subbarao *et al.,* 1993) and a Glu-92 in the NS1 protein, that is thought to suppress the antiviral response in the host (Seo *et al.,* 2002).

Formatted: Indent: First line: 35.4 pt

Discussion

The clinical and pathological signs observed in this outbreak are in keeping with previous reports, underlining the clinical susceptibility of galliform birds to HPAI. Moreover, data collected from ostriches highlights their unusual behaviour to HPAI infection, including the H5N1 virus. Other reports of natural and experimental infection (Manvell *et al*., 1998; Capua *et al.*, 2000; Olivier, 2006) had already described the total or partial resistance of these birds to clinical disease, despite the virulence of the strain, as assessed conventionally in chickens. Ostriches could therefore play a role in AI epidemiology in Africa, acting as healthy carriers of H5N1.

Deleted: -
Formatted: English (U.K.)
Formatted: Normal
Deleted:
Deleted: also with

Based on the data generated from this investigation it is not possible to make any speculations on the means by which the virus has reached the African continent. This is partly due to the high degree of homology of H5N1 contemporary isolates, which is a result of the rapid spread

Formatted: Indent: First line: 35.4 pt

For Peer Review Only

of infection over vast distances. The contemporary presence of virtually identical H5N1 viruses of the Qinghai lineage in wild and domestic fowl in Asia, Europe and the Middle East does not allow any significant conclusions on the epidemiological links that could connect Africa to Eurasia to be drawn. The debate on whether primary introduction was a result of spread from transcontinental migratory wild birds or through the movement of poultry commodities remains unclear. The index farm had poor biosecurity measures in place (free-range farming of some species and untraceable introduction of live poultry) and could have therefore contracted infection either way.

Deleted: to draw

Acknowledgements

The authors gratefully acknowledge Ron A. M. Fouchier, National Influenza Center, Virology Department, Erasmus Medical Center (Rotterdam, The Netherlands) for kindly supplying part of the primers used in this study.

For Peer Review Only

References

Adeyefa, C.A., Quayle, K., & McCauley, J.W. (1994). A rapid method for the analysis of influenza virus genes: application to the reassortment of equine influenza virus genes. *Virus Research, 32,* 391-399.

Alexander, D.J., & Spackman, D. (1981). Characterization of influenza A viruses isolated from turkeys in England during March-May 1979. *Avian Pathology, 10,* 281-293.

Becker, W.B. (1967). Experimental infection of common terns with Tern virus: influenza virus A-Tern-South Africa-1961. *The Journal of Hygiene, 65,* 61-65.

Capua, I., Mutinelli, F., Bozza, M.A., Terregino, C., & Cattoli, G. (2000). Highly pathogenic avian influenza (H7N1) in ostriches *(Struthio camelus). Avian Pathology, 29,* 643-646.

EC (1992). Council directive 92/40/EEC of 19 May 1992 introducing Community measures for the control of avian influenza. *Official Journal of European Commission*, *L 167, 22.6.1992,* p.1.

Manvell, R.J., English, C., Jorgensen, P.H., & Brown, I.H. (2003). Pathogenesis of H7 influenza A viruses isolated from ostriches in the homologous host infected experimentally. *Avian Diseases, 47,* 1150-1153.

Olivier A. J. (2006). Ecology and epidemiology of avian influenza in ostriches. Proceedings of the OIE/FAO International Conference on Avian Influenza. Schudel A., Lombard M. (eds) *Developments in Biologicals, 124,* 51-57.

Seo, S.H., Hoffmann, E., & Webster, R.G. (2002). Lethal H5N1 influenza viruses escape host anti-viral cytokine responses. *Nature Medicine, 8,* 950-954.

Spackman, E., Senne, D.A., Myers, T.J., Bulaga, L.L., Garber, L.P., Perdue, M.L., Lohman, K., Daum, L.T., & Suarez, D.L. (2002). Development of a real-time reverse transcriptase PCR assay for type A influenza virus and the avian H5 and H7 hemagglutinin subtypes. *Journal of Clinical Microbiology*, *40,* 3256-3260.

Formatted: Indent: Left: 0 pt, Hanging: 28.35 pt

For Peer Review Only

Subbarao, E.K., London, W., & Murphy, B.R. (1993). A single amino acid in the PB2 gene of influenza A virus is a determinant of host range. *Journal of Virology, 67,* 1761-1764.

For Peer Review Only

Table 1: *Primers used for polygenic sequencing.*

Deleted: Page Break

Formatted: Font: Italic

Formatted Table

Gene target	Primers	Sequence 5'-3'	References
PB2	V1F	GCAGGTCAAATATATTCAATATGG	Adeyefa *et al*., 1994
	RF263	AGTAGAAACAAGGTCGTTTTTAAAC	Provided by R. A. M. Fouchier*
PB1	V2F	CATTTGAATGGATGTCAATCCGAC	Adeyefa *et al*., 1994
	RF380	AGTAGAAACAAGGCATTTTTTC	Provided by R. A. M. Fouchier*
PA	V3F	GCAGGTACTGATCCAAAATGGAAGA	Adeyefa *et al*., 1994
	RF388	AGTAGAAACAAGGTACTTTTTTGGAC	Provided by R. A. M. Fouchier*
HA	H5startF	GGTMTAAWCTGTCAAAATGG	This paper
	H5stopR	GAACTCACAAATTTAAATGC	This paper
NP	V5NPF	AGCAAAAGCAGGGTAGAT	Adeyefa *et al*., 1994
	RF267	AGTAGAAACAAGGGTATTTTTC	Provided by R. A. M. Fouchier*
NA	N1-1F	ATGAATCCAAATCAGAAGAT	This paper
	N1-1354R	CAAACTACTTGTCAATGGTG	This paper
MA	V7F	GCAGGTAGATATTGAAAGATGAG	Adeyefa *et al*., 1994
	RF269	AGTAGAAACAAGGTAGTTTTTTACTC	Provided by R. A. M. Fouchier*
NS	NS1F	GTGACAAAAACATAATGGATTCCAAC	This paper
	NS1R	TCATTAAATAAGCTGAAACGAGAAAG	This paper

* National Influenza Center, Virology Department, Erasmus Medical Center, Rotterdam, The Netherlands

For Peer Review Only

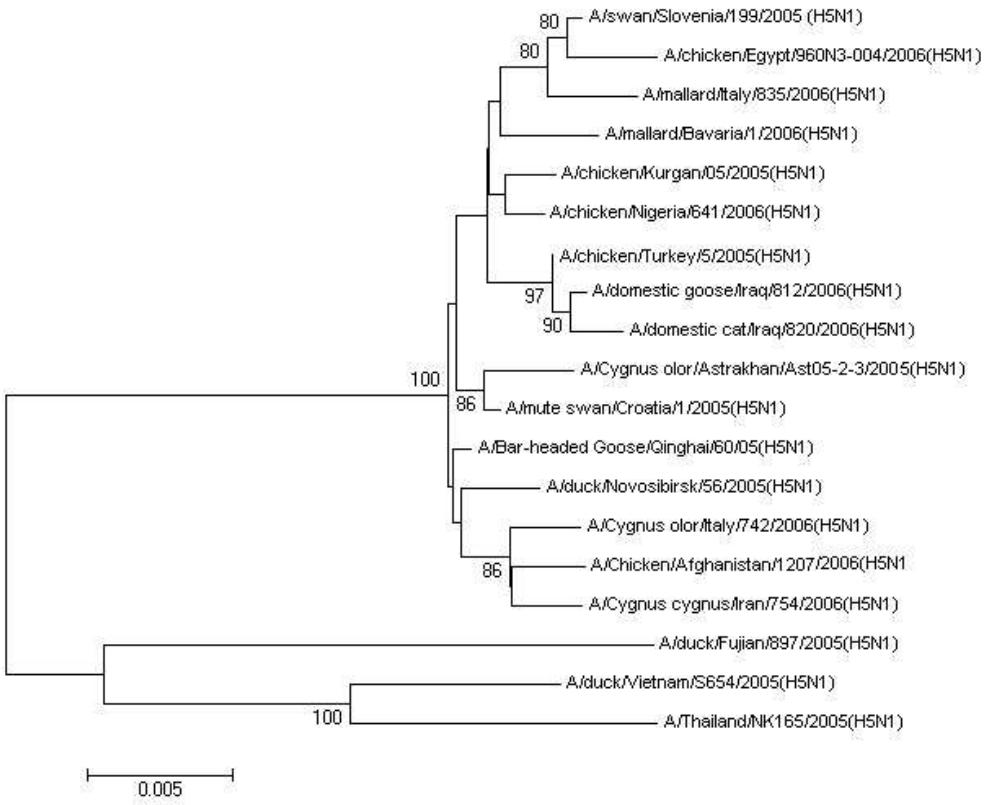

Figure 1: Phylogenetic tree based on HA nucleotide sequences of H5N1 strains. The tree, based on distance matrix, is generated by neighbour-joining algorithm within the Mega 3.1 package. The bootstrap values adjacent to the main node represent the percentage of 1000 tree that support the clustering.

For Review Only