Analysis of the PB2 gene reveals that Indian H5N1 influenza virus belongs to a mixed-migratory bird sub-lineage possessing the amino acid lysine at position 627 of the PB2 protein


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Received 28 October 2006; Accepted 27 April 2007; Published online 11 June 2007 © Springer-Verlag 2007

Summary

Outbreaks of highly pathogenic avian influenza (HPAI) H5N1 virus were reported for the first time in India during February 2006. Herein, we have sequenced and analyzed the PB2 genes of five influenza virus isolates obtained from three affected states (Gujarat, Madhya Pradesh and Maharashtra) in India during the outbreaks. In the phylogenetic analysis, the Indian isolates were grouped in the mixed-migratory bird sub-lineage of the Eurasian lineage. From the phylogenetic tree, it is evident that viruses were probably introduced to India from China via Europe because they share a direct ancestral relationship with the Indian isolates. The virus might have spread through migratory waterfowls that survived the HPAI H5N1 infection. These viruses were able to replicate in cultured cells of avian and mammalian hosts and possess lysine at position 627 of the PB2 protein, indicating that they might be able to cross the host barrier to infect mammals.

Introduction

Influenza viruses are members of the family Orthomyxoviridae and have negative-sense, single-stranded, segmented RNA in an enveloped virion [10]. The genomes encode at least 10 proteins, from the 8 RNA segments, which include two surface glycoproteins (haemagglutinin [HA] and neuraminidase [NA]), nucleoprotein (NP), three polymerase proteins (PB2, PB1 and PA), two matrix (M1 and M2) proteins and two non-structural (NS1 and NS2) proteins. Influenza viruses are classified as types A, B and C based on the antigenic properties of their nucleoprotein and matrix (M1) protein. Avian influenza is caused by type A virus, which is further classified into subtypes based on the HA and NA glycoproteins. To date, globally, 16 HA (HA1-16) and 9 NA (NA1-9) subtypes have been detected in different combinations [6, 20]. In general, wild aquatic birds serve as the natural reservoir for avian influenza viruses, where the virus remains genetically stable and helps in propagating the virus to other hosts including domestic poultry and pigs. Highly pathogenic avian influenza (HPAI) virus subtype H5N1 virus was first isolated from geese in China during 1996 [21]. Subsequently,
the virus was found to cause serious losses for the poultry industry, and since 1997 it has been reported to cause human deaths [1, 14, 18].

Control of avian influenza is dependent on effective surveillance, efficient vaccination and destruction of infected populations. Development of a regional vaccine requires knowledge of the viral variant strains circulating in the target population. In India, avian influenza virus (AIV) surveillance has been continuing since 2001, and this laboratory acts as a “National Referral Facility” for avian influenza in this country. In February 2006, an avian influenza outbreak due to H5N1 infection in poultry was reported in the state of Maharashtra, western India [13]. Subsequently, it was reported in Gujarat and Madhya Pradesh, neighboring states of Maharashtra. Preliminary HA gene-based phylogenetic analysis of the isolated virus revealed genetic similarity with two swan isolates from Iran and Italy (A/Cygnus cygnus/Iran/754/06 and A/Cygnus olor/Ireland/742/06) [13]. The two outbreak strains (Ck/India/7972/06 and Ck/India/8824/06) showed a nucleotide divergence as high as 3.3 and 1.2% in the HA1 and 2 regions, respectively, with the above two swan isolates. The infection was contained by culling tens of thousands of birds in the area within 10 km radius.

The viral polymerase consists of three subunits, polymerase acidic (PA), polymerase basic (PB) 1, and PB2 [5]. Previous reports have established the role of the PB2 protein in determining influenza virus host range and virulence. A single-gene reassortant virus that derived its PB2 gene from an avian influenza (A/Mallard/NY/78) virus and the remaining genes from a human influenza virus (A/Los Angeles/2/87) revealed that a substitution from glutamic acid to lysine (E → K) at position 627 of PB2 helps the virus to replicate in mammalian hosts. Consequently, 627 of PB2 is a major determinant of replication efficiency of the virus, and the presence of lysine at this position is indicative of adaptation to mammalian hosts.

In this study, we have determined the nucleotide sequence of the coding region (approx. 2229–2256 nts) of the PB2 genes of 5 AIV H5N1 isolates recovered from outbreaks in India. The resulting sequences and other 62 sequences obtained from the international DNA databanks were compared. The reference sequences included in the analysis, obtained from the NCBI database, were selected based on BLAST search. The influenza sequence database is growing at a rapid pace, but to our knowledge, there are no sequence data available for H5N1 virus from South Asian countries including India. Hence, the present study is one step forward in this direction.

Materials and methods

Viruses and cells

The H5N1 viruses used in this study were isolated from cloacal/nasal swabs of chickens submitted for diagnosis and are available in the virus repository of this laboratory. These viruses were used in the form of chorio-allantoic fluid collected from infected embryonated chicken eggs. The viruses were subtyped using standard serological and molecular tests recommended by OIE and WHO (details not shown). Details of the five AIV H5N1 isolates used in this study are listed in Table 1. Madin-Darby canine kidney (MDCK) cells and the African green monkey epithelial cell line Vero were grown in Glasgow modified essential medium (GMEM; Sigma, USA) containing 10% fetal bovine serum (FBS; HyClone, USA). Chicken embryo fibroblast (CEF) cells were produced by digesting pieces of 11-day-old chicken.

Table 1. History of the avian influenza virus isolates used in the study

<table>
<thead>
<tr>
<th>Isolate no.</th>
<th>Place of outbreak</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/Ck/Navapur/India/7972/06</td>
<td>Navapur, Nandurbar, Maharashtra</td>
</tr>
<tr>
<td>A/Ck/Surat/India/9256/06</td>
<td>Surat, Gujarat</td>
</tr>
<tr>
<td>A/Ck/Jalgaon/India/13840/06</td>
<td>Jalgaon, Maharashtra</td>
</tr>
<tr>
<td>A/Ck/Thane/India/15053/06</td>
<td>Thane, Maharashtra</td>
</tr>
<tr>
<td>A/Ck/Indore/India/18760/06</td>
<td>Indore, Madhya Pradesh</td>
</tr>
</tbody>
</table>

* The isolate number consists of influenza virus type/host species/place of outbreak/country of isolation/isolate number/year of isolation. Ck Chicken.