2004 年山东分离的一株 H5N1 禽流感病毒被检测为 X 基因型

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摘 要: A / Duck / Shandong / 093 / 2004 被报道是一株重配的 H5N1 禽流感病毒.为此, 对它进行了基因型研究. 对其 8 个独立的基因片段的贝叶斯分析和对全基因组的邻接法分析都表明它属于基因型 X, 尽管基因型 X 在中国南部在 2002 年已被基因型 Z 所取代.

关键词: H5N1; 禽流感病毒; 基因型; 重配; 山东

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An X Genotype H5N1 Virus was Identified in Shandong in 2004

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Abstract: A / Duck / Shandong / 093 / 2004 was reported to be a reassorted H5N1 avian influenza virus.A phylogenetic study of it was conducted to identify its genotype.A Bayesian phylogenetic analysis of eight separate gene segments and a neighbor-joining analysis of its whole genome demonstrated that it was an the X genotype virus, although this genotype was reported to be replaced by genotype Z in Southern China in 2002.

Key words: H5N1; avian influenza virus; genotype; reassortment; Shandong

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Influenza viruses are negative-strand RNA viruses of the Family Orthomyxoviridae.They infect a wide range of warm-blooded animals including domestic and wild birds and mammals.They pose significant threats to animal health and cause a huge economic loss every year.To date, more than ten people have died because of infection with highly pathogenic avian influenza viruses in China[1].

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Influenza viruses are able to acquire new genetic or antigenic features by means of genetic reassortment with prevailing influenza viruses[2]. Single or multiple reassortment events may allow them to escape recognition by the host's immune system. As a result, older vaccines may be inefficacious. Thus, tracing the evolution and variance of influenza viruses is vital to prevention and development of vaccine.

Frequent reassortment has been detected in recent years all through the world[3,4]. Several genotypes of H5N1 avian influenza viruses have been detected in the past five years, and they were designated A, B, C, D, E, G, V, W, X, Y, Z and Z+[5~8] respectively. H5N1 viruses of genotype Z became leading in Southeast Asia and Southern China in 2002, while other genotypes have died out gradually. It was H5N1 highly pathogenic viruses of genotype Z that were responsible for the regional outbreaks in 2003~2004[8].

A lot of genotype analyses have been done in the provinces in southern China, especially in Hong Kong Special Administration Region (SAR). However, none was conducted for the avian influenza viruses isolated in Shandong Province before. Here we present a genetic study of an H5N1 isolate from Shandong in 2004. We found it was an X genotype H5N1 highly pathogenic avian influenza virus.

1 Materials and methods

1.1 Materials

The Shandong isolate was A/Duck/Shandong/093/2004 (Dk/SD/093/04). This isolate was collected and sequenced by Long, et al.[9]. The GenBank database was searched using the nucleotide sequence of each segment from the Shandong isolate and the Basic Local Alignment Search Tool (BLAST)[10]. Datasets were compiled for the eight influenza genes from sequences that were closely related to the Shandong sequences and representatives of lineages that were closely related to those sequences. Without a few exceptions, lineages were included when BLAST alignments showed that the nucleotide sequences were more than 90% identical to the Shandong isolate.The known reassortant H5N1 genotypes that were represented in the datasets included A, B, C, D, E, G, W, X, Y, Z and Z+. Note that all the DNA data are obtained from GenBank directly.DNA data were translated to their protein sequences by using Mega 3[11].

1.2 Methods

Multiple sequence alignment was performed with ClustalX 1.81[12] for the major coding regions of the eight single genes and the alignment parameters were set to default. To estimate the trees accurately, MrBayes, version 3.0b4[13] was used to analyze the eight individual gene segments respectively. Four Markov chains were run for two million generations and sampled every 100 generations to yield a posterior probability distribution of 20 000 trees. After eliminating the first 5 000 trees as burn-in, a 50% majority-rule consensus tree was constructed. Bayesian Posterior Probability(BPP) was used to assess the support for the recovered clades, given the aligned sequence data. A six parameter substitution model was used with a gamma rate parameter allowing site variation.

The whole genome was constituted by concatenating the alignments of the eight major coding regions. Neighbor-joining (NJ) method was implemented for the whole genome using Mega 3[11]. In NJ analysis, gaps were scored as pairwise deletions and all variable characters were weighted equally. The Tamura-Nei-γ model was adopted to search for the NJ tree. One thousand bootstrap replicates were conducted to test the phylogeny.

2 Results

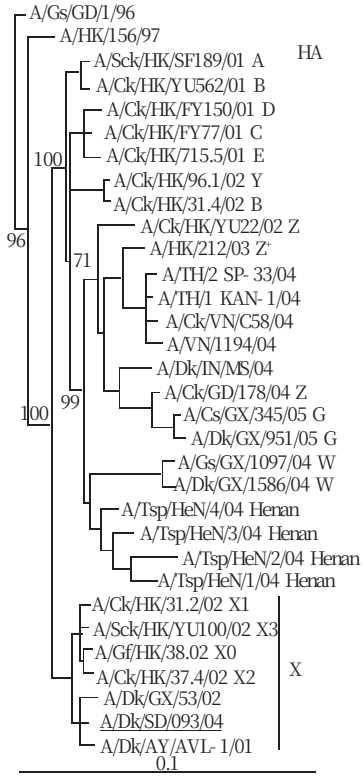
2.1 Bayesian analysis

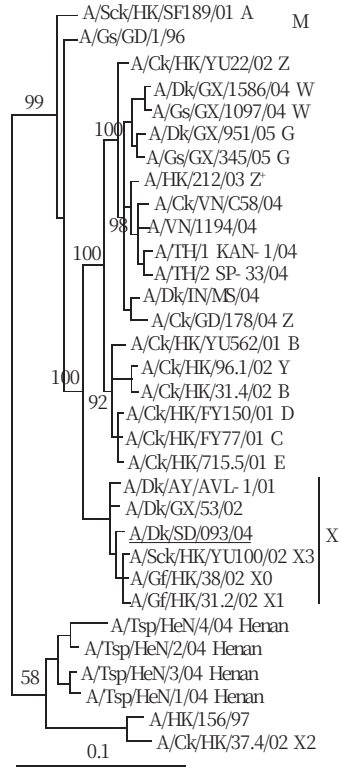
In the gene tress of hemagglutinin(HA), neuraminidase(NA) and nonstructural(NS), all the X genotype isolates formed a separate sublineage and Dk/SD/093/04 was placed within this sublineage. For the gene trees searched using the matrix(M), nucleoprotein(NP), polymerase acidic(PA), polymerase basic 1(PB1) and polymerase basic 2(PB2) gene segments, one or two isolates of X genotype dispersed into these trees. However, most of the X genotype isolates

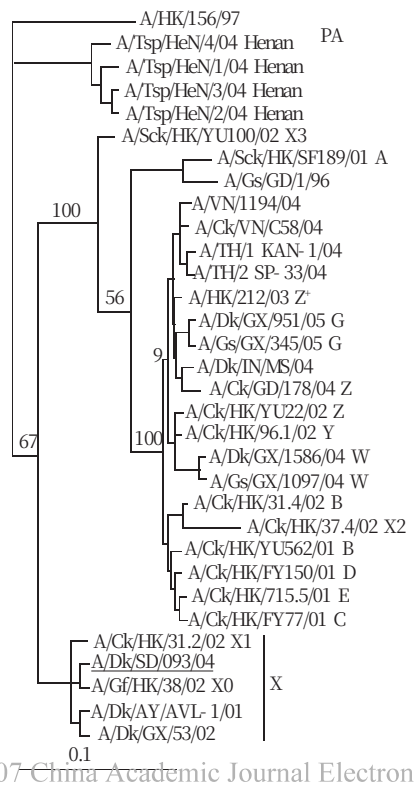
still clustered together and constructed an independent clade. In addition, Dk/SD/093/04 was placed into these clades without any exception(Fig.1) .

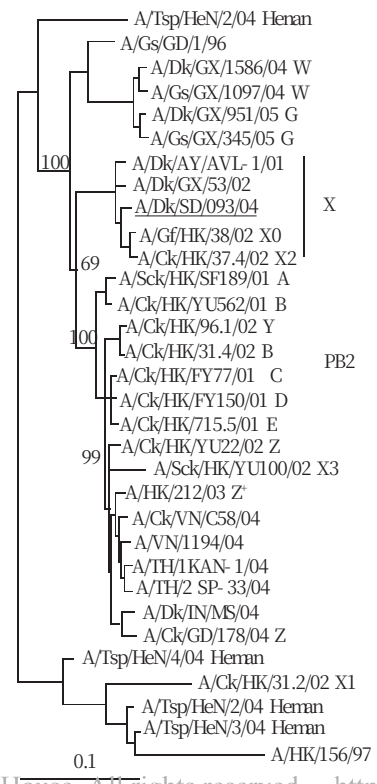
2.2 NJ analysis

The NJ analysis supported the phylogenetic relationships found in the Bayesian analysis(Fig.2) . Isolates of all the X genotype, including Dk/SD/093/04, clustered within an independent sublineage in the gene tree built by the whole genome.









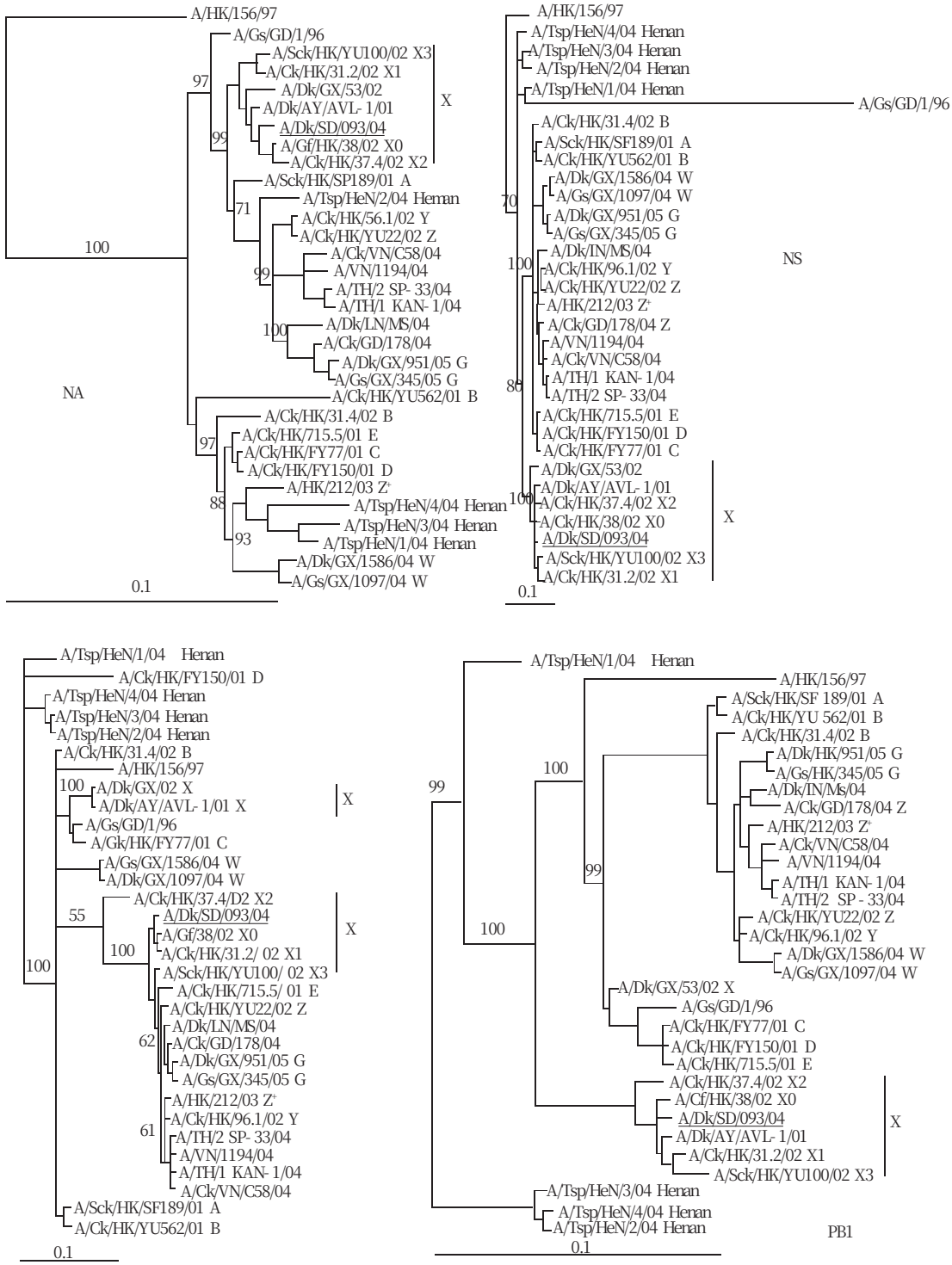


Fig.1 Phylogenetic relationships of the individual major coding regions of representative H5N1 viruses of various genotypes

Numbers below or above branches indicated BPP values. Underlined viruses was isolated from Shandong in 2004.Analysis was based on nucleotides 29-1720 (1 692 bp) of HA gene(including the signal peptide), 26-1003(978 bp) of M gene, 21-1367(1 347 bp) of NA gene, 46-1536(1 491 bp) of NP gene, 27-842(816 bp) of NS gene, 25-2172(2 148 bp) of PA gene, 25-2283 (2 259 bp) of PB1 gene and 28-2304 (2 278 bp) of PB2 gene, numbering according to A/Dk/SD/093/04. Outgroup for M, NS, NP and PB2 was A/Equine/Prague/1/56.Outgroup for HA was A/Tern/South Africa/61. The NA tree was rooted to A/Parrot/Ulster/73, while the PA and PB2 trees were rooted to A/Ann-arbor/6/60. Scale bar, 0.1 nucleotide change per site. AY, Anyang; Ck, chicken; Dk, duck; GD, Guangdong; Gf, Guinea fowl; Gs, goose; GX, Guangxi; HeN, Henan; HK, Hong Kong; IN, Indonesia; Sck, silky chicken; SD, Shandong; TH, Thailand; Tsp, tree sparrow; VN, Vietnam.

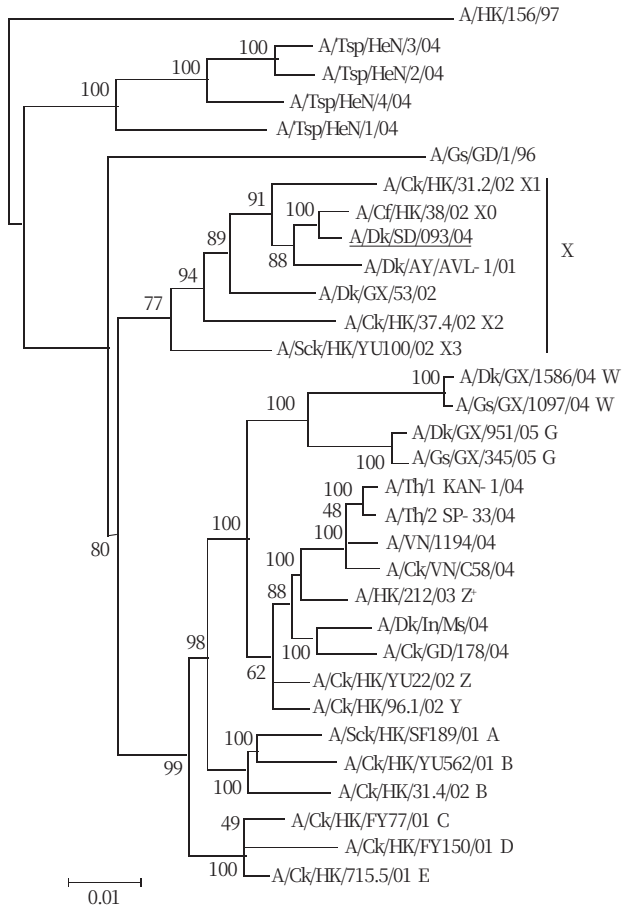


Fig.2　Phylogenetic relationships of the whole genomes (concatenated major coding regions) of representative H5N1 viruses of various genotypes

Underlined virus was isolated from Shandong in 2004. Numbers below or above branches indicate bootstrap values from 1 000 replicates. Scale bar, 0.01 nucleotide change per site. AY, Anyang; Ck, chicken; Dk, duck; GD, Guangdong; Gf, Guinea fowl; Gs, goose; GX, Guangxi; HeN, Henan; HK, Hong Kong; IN, Indonesia; Sck, silky chicken; SD, Shandong; TH, Thailand; Tsp, tree sparrow; VN, Vietnam.

3　Discussion

Dk/SD/093/04 was reported by Long et al. for the first time[9]. Molecular characterization analysis revealed that the motif in the connecting peptide accorded to the typical motif R-X-K/R-R of a highly pathogenic avian influenza virus[14,15]. In addition, Long et al. believed that it was a reassorted virus based on the phylogenies. However, their analyses had not included enough samples and did not identify its genotype exactly[9]. In this paper, sequences of more H5N1 genotypes were represented included into our analyses and it was identified to be an H5N1 avian influenza virus of the X genotype.

X_0 genotype was isolated before 2002. From 2002 onwards, three new genotypes similar to X_0 were detected and designated as X_1, X_2, X_3 respectively[8]. Not clustering together within a separate sublineage for all the eight major coding regions suggested the differences among these four X genotypes (Fig.1) . X genotype viruses distributed in southern China extensively in 2001~2002, such as Hong Kong SAR, Guangxi, Shantou, Henan, and so on. However, X genotype, together with genotype A-E and Y, was replaced by genotype Z viruses in a short time in 2002[8]. For example, X genotype viruses disappeared and Z and W genotypes became dominant in Guangxi in 2004[16], while X genotype viruses were taken place by H5N1 viruses of an unnamed genotype in Henan at the same time[7].

However, our data supported that Dk/SD/093/04 was a reassortant virus and it belonged to the X genotype. It suggested that X genotype viruses were still circulating in Shandong in 2004.Thus, the avian influenza viruses of Shandong might have been under less natural selection pressure.Moreover, as a

province with a flourishing animal husbandry [17], Shandong exported a lot of poultry products annually. This unilateral transportation of poultry also might have reduced the possibility of viruses transmitting from other places to Shandong.

Nonetheless, Shandong Province is located in the migrating route of eastern Asia-Australia [18] and therefore is a natural habitat and stopover for thousands of migratory birds. More important, it has been identified that bird migration and transport of poultry were able to transmit avian influenza viruses through a long distance[3]. In addition, avian influenza viruses of other subtype coexisting with H5N1 viruses in Shandong for a long time makes it possible to produce new reassorted viruses[19~23]. Therefore, although no occurrence of highly pathogenic H5N1 viruses has been reported in Shandong before, it is still very important to surveil both the migratory birds and the poultry transportation. In particular, injecting vaccines to healthy poultries in advance is strongly recommended.

It is also noteworthy that although it was reported some genotypes, such as X, W, have been replaced in 2002 in some provinces of southern China[8], they were still detected in other areas in China after 2002[16]. From this point, it might be better to study the evolution of local avian influenza viruses before selecting vaccines.

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