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Molecular epidemiology and evolution of avian infectious bronchitis virus in Spain over a fourteen-year period

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Abstract

An in-depth molecular study of infectious bronchitis viruses (IBV) with particular interest in evolutionary aspects of IBV in Spain was carried out in the present study based on the *S1* gene molecular characterization of twenty-six Spanish strains isolated over a fourteen-year period. Four genotypes were identified based on *S1* gene sequence analyses and phylogenetic studies. A drastic virus population shift was demonstrated along time and the novel Italy 02 serotype was shown to have displaced the previous predominant serotype 4/91 in the field. Detailed analyses of synonymous to non-synonymous ratio of the *S1* gene sequences of this new serotype Italy 02 suggested positive selection pressures might have contributed to the successful establishment of Italy 02 serotype in our country. In addition, differences on the fitness abilities of new emergent genotypes were indicated. Furthermore, intergenic sequences (IGs)-like motifs within *S1* gene sequences of IBV isolates were suggested to enhance the recombination abilities of certain serotypes.

Keywords: Infectious bronchitis virus; Genotyping; *S1* gene; Selection pressures; Phylogeny; Coronavirus; Recombination; Virus neutralization; Evolution; Sequencing

Introduction

Avian infectious bronchitis virus (IBV) is considered one of the major poultry pathogens, being probably endemic in all regions with intensive poultry production (Office, 2004). It is primarily a respiratory pathogen of domestic fowl, although strains can vary in pathogenicity and tissue tropism (Jones and Ambali, 1987; Jones and Jordan, 1970; Winterfield and Hitchner, 1962). IBV is a Group 3 member in the genus Coronavirus (Gonzalez et al., 2003) and its genome consists of a 27 Kb single-stranded positive-sense RNA molecule that encodes for four structural proteins. The nucleoprotein (N) is associated with viral genome to form the nucleocapsid, whereas the remaining structural proteins, the spike (S), small membrane (M), and envelope proteins (E), are inserted in the envelope surrounding the nucleocapsid. In addition, the genome also encodes for the replicase complex responsible for carrying out the unique discontinuous transcription process that leads to the generation of a nested set of six 3′ and 5′ coterminal subgenomic mRNAs (Stern and Kennedy, 1980; Stern and Sefton, 1982).

The continuous emergence of new IBV serotypes has complicated the design of appropriate control programs due to the antigenic variation and the low degree of cross protection observed among IBV serotypes and has pointed out the necessity for accurate techniques to diagnose and classify this viral agent (Cowen and Hitchner, 1975). Nucleotide sequencing and subsequent genetic analysis of the S1 protein gene sequences provides a fast and accurate method to classify and predict IBV serotype, but also a powerful instrument to monitor phylogenetic and epidemiological evolution of IBV subtypes (Adzhar et al., 1997; Cavanagh and Davis, 1986; Cavanagh et al., 1992b, 1986; Kant et al., 1992; Koch et al., 1990; Lee and

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Jackwood, 2001; Liu et al., 2006; Mase et al., 2004; Moore et al., 1998; Wang and Huang, 2000). Mutation and recombination processes have been demonstrated to be involved in the genetic variation, and therefore in the evolution of IBV, leading to the emergence of new variant strains and giving rise to virus population diversity (Cavanagh et al., 1992a; Kottier et al., 1995; Lee and Jackwood, 2000; Wang et al., 1997). However, very distinct spatiotemporal and epidemiological behaviours are shown among these new strains. Whereas some emergent viruses rapidly spread to other geographic areas and become established, as happened with the 4/91 serotype in Europe or the Georgia serotype in North America, others remain restricted to the region of origin. More interestingly, hardly ever genotypes are exchanged among different continents (Kusters et al., 1987; Zanella et al., 2003). Nevertheless, factors that determine distinct spreading and fitness abilities of the new emergent strains are poorly understood. Hence, monitoring IBV subpopulation dynamics in a specific region over time may reveal molecular features that contributed to the different fitting and spreading abilities of IBV strains.

IBV has been diagnosed in Spain since the early seventies by virus isolation and serological techniques. In 2002, an epidemiological survey among the most important Spanish poultry companies pointed out IB as one of the main infectious diseases affecting farms and revealed the necessity of an in-depth study to determine the epidemiological situation and to improve the efficacy of vaccination and control programs against the disease. Furthermore, the availability of IBV strains isolated in Spain during the past 14 years provided a unique opportunity for a detailed genetic study to determine not only the prevalent IBV genotypes in our country but also the dynamics of viral subpopulation which is necessary for a deeply understanding of IBV evolution and epidemiology.

In this study, twenty-six IB viruses isolated in Spain from clinical outbreaks occurring between 1992 and 2005 were molecularly characterized by sequencing the whole *S1* gene. To increase our insight into factors involved in IBV evolutionary process, specific tests to determine the presence of positive selection forces were carried out. Furthermore, several methods were implemented to identify putative recombination events among these isolates and establish the extent of recombination into IBV evolution. Finally, the antigenic relationships of the major genotypes identified in our country with reference strains were assessed by cross VN assays to ascertain the practical implications of molecular changes observed into virus immunogenicity.

Results

S1 gene phylogenetic and sequence analyses revealed that four different genotypes have been present in Spain over the last 14 years

Phylogenetic analysis based on both *S1* gene nucleotide and amino acid deduced sequences of the twenty-six Spanish isolates and the reference IBV strains showed that Spanish isolates were separated into four distinct genetic groups or genotypes (Fig. 1). Genotype I included thirteen out of the twenty-six field isolates, isolated between 1992 and 2000, that were grouped with 4/91 reference isolates. Seven Spanish field isolates, from 1997 to 2005, were included in genotype II, and showed maximum nucleotide and amino acid identities with Italy 02 strains. Genotype III comprised three field viruses isolated between 1996 and 2000 that were grouped with isolates of the Massachusetts serotype. Particularly, Spain/98/308 and Spain/00/339 isolates were grouped with Massachusetts vaccine strains (H120) in the phylogenetic tree, and they are likely to be reisolations of vaccine strains used in the immunization of those flocks. Finally, Spanish genotype IV included 3 isolates that split in a unique genetic group in the phylogenetic analysis. All viruses included in genotype IV were isolated in 1999 and showed maximum nucleotide and amino acid identities with Italy 02 isolates (90.6–92% and 89–91.1% respectively).

Genetic studies of Spanish isolates of 4/91 genotype (genotype I) showed the presence of two distinct genetic clusters

The 13 isolates assigned into the Spanish genotype I group shared maximum nucleotide and amino acid divergences of 7.3% and 10.3% respectively. Phylogenetic analyses comparing the complete *S1* gene sequences of the 13 Spanish genotype I field isolates and all 4/91 *S1* gene sequences previously published in the GenBank database, including British, French and Iranian isolates, revealed that Spanish isolates were clearly grouped in two separated genetic clusters identified as Spanish genotype I cluster 1 (including 11 isolates) and Spanish genotype I cluster 2 (including 2 isolates). Average nucleotide and amino acid identities shared by both clusters were 93.1% and 91.3% respectively. The minimum amino acid divergence observed between two isolates from the same year was 0.4% (between Spain/92/185 and Spain/92/51), whereas the maximum was 9.9% (between Spain/99/319 and Spain/99/327). Both cluster 1 and cluster 2 isolates showed maximum average nucleotide and amino acid identities with French isolate FR-94047-94. Comparison of 4/91 Spanish field isolates sequences revealed a common unique deletion at amino acid position 58 (located in HVR1) in 6 isolates included in cluster 1. Four isolates within this same group shared an amino acid deletion at position 340, located in HVR3. Both isolates included in cluster 2 showed 8 unique amino acid substitutions. In addition, isolate Spain/99/327 in cluster 2 showed 3 amino acid deletions at positions 55, 56 and 57 (located in HVR1). No cumulative changes were observed among 4/91 Spanish isolates.

Sequence analysis of Spanish isolates of Italy 02 genotype (Genotype II) clearly revealed presence of cumulative amino acid changes within S1 gene

A total of 11 Spanish field strains of Italy 02 genotype, seven from the present study and 4 previously described (Dolz et al., 2006), and the reference strain Italy 02 were included in the present study. Maximum nucleotide and amino acid divergence within Spanish Italy 02 isolates was 5.7% and 9.9%, respectively. The maximum amino acid distance between two viruses isolated the same year was 3.6% (Spain/04/5438 and Spain/04/