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Evolution of avian infectious bronchitis virus: Genetic drift and recombination

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Abstract

Infectious bronchitis(IB) is a viral disease in which continued evolution of the virus is of paramount importance for annual endemics and epidemics in chickens. Since the isolation of IB viruses(IBVs) in the 1930s, over 50 serotypes or variants have been reported worldwide. Continuing evolution is most prominent in the surface glycoproteins of IBV but also occurs in other parts of the genome. This genetic variability results from accumulation of molecular changes that can occur by a number of different mechanisms including genetic drift (point mutations) and genetic shift(RNA recombination). GA98 is a new serotype of IBV identified recently in the United States. In this paper, the evolutionary trend of IBV will be discussed using GA98 serotype as a model.

Key words : Infectious bronchitis virus, Evolution, Genetic drift, Recombination

Introduction

Infectious bronchitis(IB) is an acute, highly contagious respiratory disease of chickens caused by infectious bronchitis virus(IBV)[1]. IBV is classified as a member of the new order Nidovirales in the family coronaviridae[2]. The enveloped virus has a positive-sense single stranded RNA genome of approximately 27 kilo-bases, which codes for an RNA-dependent RNA polymerase, spike (S), gene 3a, 3b, envelope(E), membrane, gene 5a, 5b, and nucleocapsid protein (N) in the 5′ to 3′ direction[3]. IB is one of the major respiratory problems in the poultry industry and the difficulty in control of the disease is due to the continued evolution of the IBV. Virus evolution in IBV can be observed through the occurrence of variants or new serotypes of IBV. Since the isolation of IBV in the 1930s, over 50 serotypes or variants have been reported worldwide[1]. In the United

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States, the Massachusettes strain was the first serotype identified and has been the most prevalent. However, isolation of new variant IBV continuously occurred, and serotypes other than Massachusettes, such as Connecticut, Arkansas, and DE072 are now common. Continuing evolution is most prominent in the surface glycoproteins of IBV but also occurs in each of the individual genes. The variability results from accumulation of molecular changes in the viral RNA that can occur by a number of different mechanisms including point mutations, RNA recombination, and defective-interfering particles[4]. Each of these mechanisms may contribute to the evolution of IBV.

We previously identified GA98, a new serotype of IBV that was derived from the DE072 serotype[5]. The GA98 serotype isolates are an excellent choice of viruses for studying the evolution of IBV because they are very unique in their genome sequence compared to other serotypes of IBV and can be easily differentiated from vaccine strains including DE072 vaccine. Further, they have persisted in the environment long enough to determine an evolutionary trend. In this paper, we will discuss the trend and importance of genetic drift and shift occurring in IBV using GA98 as a model virus.

Materials and Methods

Sequences

Published sequences were obtained from GenBank and EMBL. For the construction of the phylogenetic tree in Figure 3, we newly sequenced the M gene and the 5′ end of the N gene of 14 isolates. Primer sequences are available upon request. The nucleotide sequences reported here have been deposited with GenBank(Accession numbers : AF363597 - AF363610).

Phylogenetic analyses

Assembly of sequencing contigs, translation of nucleotide sequence into protein sequence, and initial multiple sequence alignments were performed with the MegAlign software version 1.03(DNAStar Inc, Madison, WI). Phylogenetic trees were generated using the maximum parsimony with 100 bootstrap replicates in a heuristic search using the PAUP 4.0b software program[6].

Results and Discussion

1) Genetic and antigenic drift

Mutations, including substitutions, deletions, and insertions, are one of the most important mechanisms for producing variation in IBVs. Genetic drift is a process and result of the accumulation of mutations in viral genes. The lack of proofreading among RNA polymerases contributes to replication errors on the order of 1 in 10^4 bases. Since the IBV genome contains approximately 30,000 nucleotides, an average of three mutations per template copied will result. So, each round of IBV replication in chickens results in a mixed population with many genetic variants. Transmission of only a few genetically different virus particles to other susceptible chickens will result in genetic drift. If the point mutation(usually non-synonymous amino acid changes) accumulates in the antigenic domain of the viral proteins, a virus with a slightly changed antigenic structure can appear. This process is called antigenic drift to differentiate it from genetic drift. Antigenic drift is well characterized in H3 influenza viruses that ap-

peared in 1968 and have accumulated approximately 7.9 nucleotide and 3.4 amino acid substitutions per year[7]. In IBV, new serotypes can arise from antigenic drift in the surface glycoprotein gene. Jackwood et al[8] demonstrated the genetic drift occurring in the Arkansas serotype of IBV. Further, the S1 glycoprotein of the CU-T2 strain carries virus-neutralizing and serotype-specific epitopes of two IBV serotypes, Arkansas and Massachusettes[9]. Sequence analysis revealed that the virus, originally an Arkansas serotype, has acquired the Massachusettes-specific epitope by mutations. This provides evidence that point mutations may lead to the generation of IBV antigenic variants in the field.

GA98 vs 793/B serotype of IBV

To determine evolutionary trend, all isolates included in the analysis must have descended from a single introduction of the virus. It is not easy to collect those isolates that persisted long enough in the field to determine a trend in IBV because of widespread vaccine use. Two serotypes of IBV, 793/B and GA98, for the most part meet these criteria. As a brief background, the 793/B serotype of IBV was first identified in 1990 in Great Britain and is distributed throughout Europe[10]. The GA98 serotype has descended from the DE072 serotype of IBV, which was isolated in 1992 in North America[5,11]. The vaccine for the DE072 serotype of IBV was introduced in 1993. We conducted extensive genetic analysis of DE072 derivatives and found several subgroups of DE072. By conducting antigenic and vaccine protection tests, one of the subgroups was designated as GA98. The 793/B and GA98 serotypes of IBV have extremely large antigenic and genetic differences with other serotypes of IBV[10,11]. The whole S1 amino acid sequence of those serotypes differ by 20 to 50% or more from that of other IBV serotypes(Fig 1). This extensive difference has probably contributed to the persistence of these two serotypes in the field.

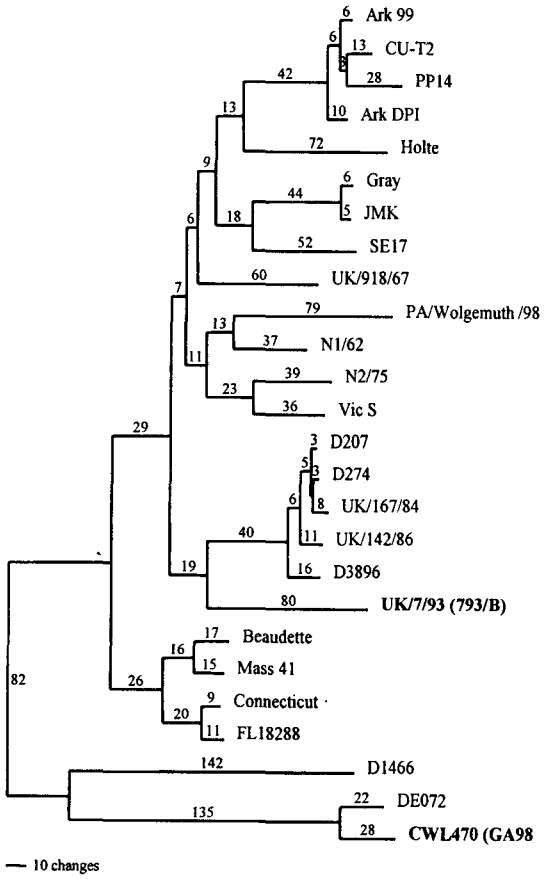

Fig 1. Phylogenetic relationships based on the amino acid sequence of the S1 gene of 26 IBV isolates. Tree was midpoint rooted and branch lengths are provided.

The nonsynonymous and synonymous changes provide useful markers in the epidemiological tracking of viruses during an outbreak. The evolutionary rate can be estimated by examining the nonsynonymous or amino acid changes that occurred in

viruses from the same lineage as sampled over time[12]. Adzhar and Cavanagh conducted a molecular analysis of the 793/B serotype and the results give a good contrast with our results done with the GA98 serotype. They analyzed the 793/B serotype of IBV obtained between the years 1985(first isolation) and 1996[10]. The estimated nucleotide mutation rate was only 0.3% to 0.6% per year indicating that this serotype was not evolving. At the time of their study, no vaccine was being used against that serotype of IBV. On the contrary, in North America DE072 vaccine was being used early in the outbreak. By examining GA98 and DE072 isolates from 1990 to 2000, we found that mutations were progressive and fixed, and the mutation rate was 1.5% per year[13]. The 793/B serotype is antigenically very different from other IBV vaccines and the lack of immune pressure should be considered when interpreting the evolution of this virus. The evolution of these two serotypes of IBV clearly shows how virus behaves against immune pressure. The difference of evolutionary trend between the 793/B serotype and GA98 is summarized in Table 1.

2) Genetic shift / Recombination

Genetic shift refers to the extensive change in viral genes by two distinct but not mutually exclusive types of genetic exchange that operate in RNA viruses : reassortment and recombination. Reassortment occurs only in multipartite viruses such as influenza virus and involves swapping one or more of the discrete RNA molecules that make up the segmented viral genome. Recombination can occur in either segmented or unsegmented viruses when donor nucleotide sequence is introduced into a single, contiguous acceptor RNA molecule to produce a new RNA containing genetic information from more than one source.

A high rate of recombination has been documented in coronaviruses and recombination between vaccine strains in the S1 gene, may create a new field variant. By conducting sequence and phylogenetic analysis, many IBV strains are thought to be a recombinant. Recombinations in the S1 gene were identified in UK682, SE17, PP14, and DE072. In the CU-T2 strain, two independent recombination events involving three different IBV strains had occurred in the S2 gene and N protein gene indicating that genomic RNA recombination in IBV may occur in multiple genes. Further, a sequence from Holland 52(a vaccine strain) had replaced half of the N gene of CU-T2 suggesting that recombination with vaccine strains is contributing to the generation of new IBV variants in the field[9,14,15].

High frequency of homologous recombination at the intergenic (IG) sequence

Due to a limited amount of sequence information, recombination has only been de-

Table 1. Comparison of GA98 and 793/B serotype of IBV

Serotype	GA98	793/B
Prevalence	North America	Europe
Immune pressure	Vaccine use	No vaccination
Trend of mutation	Progressive and fixed	Random
Mutation rate	1.5% per year	0.3% per year

scribed for a small part of the genome. Examining only a small part of the genome may result in misleading conclusions because of point mutations or conserved regions of the gene. The genome of IBV is organized into six regions, each containing one or more open reading frames, which are separated by IG sequences that contain the signal for transcription of subgenomic mRNAs. The IG sequence is a stretch of consensus sequence in IBV and the sequence around this region is highly conserved[4]. Since recombination in coronaviruses is thought to occur by a template switching mechanism, we previously proposed that IG sequences may serve as a hot spots for homologous recombination. We showed evidence of template switching by conducting phylogenetic analysis on the genome of five IBV strains, of which the complete sequence of the 3′ end of the genome has been determined. We further showed that isolates within the same serotype might have different amounts of nucleotide sequence similarity with each other in individual genes other than the S gene[16].

In addition, we confirmed the high frequency of recombination in these regions in isolates within the same serotype of IBV that were isolated in same regions by phylogenetic analysis of selected regions of the genome(S, M and N gene). In a phylogenetic tree based on the S1 gene, all the GA98 isolates cluster together in one group with the DE072 strain from which they originated. However, the phylogenetic tree of M and N genes showed differences in tree topology

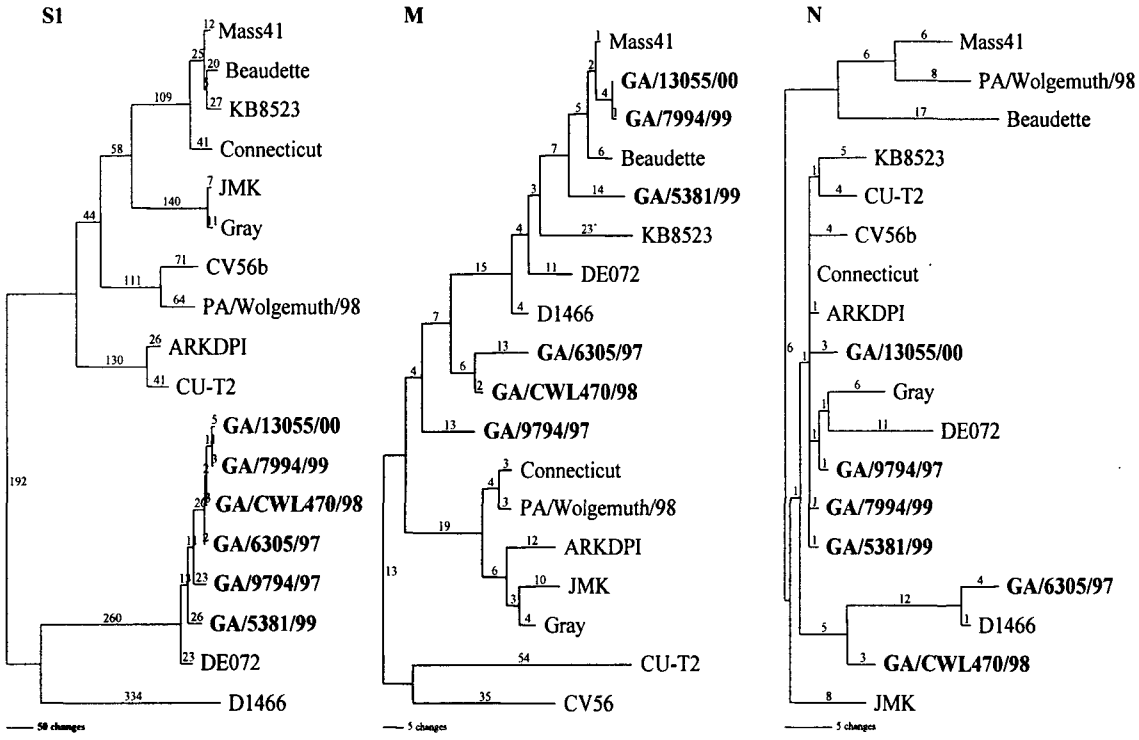

Fig 2. Phylogenetic analysis of field isolates of GA98 serotype IBV based on nucleotide sequence of S1, M and 5′ end (421bp) of the N gene. Tree was midpoint rooted and branch lengths are provided. Field strains isolated in same region are in bold characters.

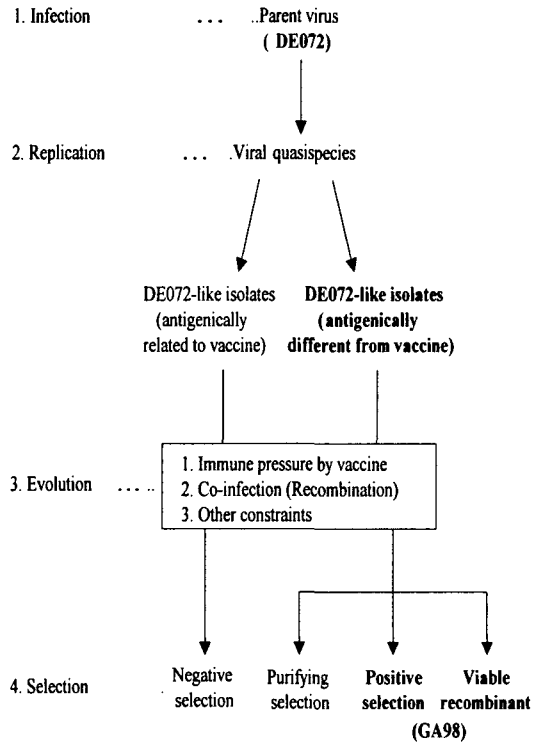

Fig 3. Schematic diagram of the possible evolutionary path and the creation of the GA98 serotype from the DE072 serotype of IBV.

among six isolates, indicating that recombinations had occurred(Fig 2).

It has been reported that RNA recombination in IBV can occur randomly in non-localized sites *in vitro*[17]. However, considering the selection pressure *in vivo*, recombination in the IG sequence should be advantageous to virus because there would be no drastic change in the conformation of proteins encoded by individual genes. Further, cross-overs at each of the five IG sequences would generate tremendous genetic diversity which may contribute to persistence and to the continuing emergence of new variants of IBV despite immune pressure by vaccination.

However, the occurrence of RNA recombination generating variant serotypes has yet to be determined. Further, it is possible that this kind of recombination in IBV may not affect the antigenic or phenotypic characteristics of the virus especially in those where recombination occurred in an area other than the S1 gene. In the context of viral evolution, recombination is a fast and efficient way to get rid of accumulated deleterious changes in the genome and to create or spread beneficial combinations of mutations in an efficient manner.

Conclusions

The primary advantage of a high error rate is the rapid adaptation to a new environment. It appears that GA98 lineage viruses fall into this category. On the contrary, the high error rate can produce many viral genotypes that are defective or have a reduced fitness for the current host. DE072-like isolates, which were isolated from different regions and did not persist in the field, may fall into this category. However, immune pressure by vaccination and a high frequency of recombination should be considered together in interpreting the evolution of this virus. Fig 3 shows a potential model for the evolution of GA98 from DE072.

Predicting whether a new variant of IBV will or will not persist in the environment is difficult. Our work highlights the effect of vaccine use on the evolutionary path of IBV and the evolutionary and mutation rates of GA98 will be useful as a reference for studying the evolution of other IBV serotypes.

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