

Genetic Relatedness of Commercial and Local 793/B-like IBV Vaccines in Iran

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Abstract

Infectious bronchitis virus (IBV), a genetically diverse Gamma coronavirus, poses a major threat to poultry health due to frequent mutations and recombination events. In Iran, 793/B-like IBV strains remain dominant despite widespread vaccination, raising concerns about vaccine-field strain compatibility. This study assessed the genetic relatedness between four 793/B-like vaccine strains—three commercial (BK-07, GI-13, A/91) and one local (793/B.08IR)—and archived Iranian field isolates. The full S1 gene was amplified and sequenced, revealing 92–100%

nucleotide identity among vaccine strains and field isolates. Commercial vaccines showed 100% identity with each other and clustered with the classic 4/91 strain, while the Razi strain matched the Iranian IB88 variant. These results confirm strong genetic conservation and relevance of current vaccines to circulating genotypes. However, minor antigenic differences in the S1 gene may affect immunity, underscoring the need for ongoing molecular surveillance to optimize vaccine efficacy and reduce IBV-related losses in Iranian poultry.

Keywords: Infectious Bronchitis Virus [IBV]; 793/B-like Vaccine; Sequence Analysis; Vaccine–field strain matching

1. Introduction

Infectious bronchitis virus [IBV] is a highly contagious pathogen in poultry, classified under the genus Gamma coronavirus within the family *Coronaviridae* [1]. It primarily infects chickens, causing respiratory, renal, and reproductive disorders that result in significant economic losses worldwide [2,3]. The virus exhibits remarkable genetic diversity, driven by high mutation rates and frequent recombination events, leading to the continuous emergence of novel genotypes and variants with distinct antigenic profiles [4,5].

Control of IBV largely depends on vaccination; however, the high level of genetic and antigenic variability among IBV strains poses a major challenge to effective immunization [6]. Cross-protection between different genotypes is often incomplete, and vaccine failures may occur when field strains differ significantly from the vaccine strains in use [7]. Therefore, selecting appropriate vaccine strains based on regional viral genotypes is critical for successful disease control.

Among the many IBV genotypes, the 793/B-like strain [also known as 4/91-like or CR88-like] is one of the most widely used vaccine backbones and has been implemented globally due to its broad protective potential [8]. In Iran, 793/B-like strains continue to circulate extensively in poultry populations, despite the application of mass vaccination programs using both imported and domestically produced vaccines [9-11]. One of the domestic vaccines is the 793/B.08IR [AREEO, Iran], a live attenuated vaccine, has been developed in Iran to combat the 793/B serotype of infectious bronchitis virus [IBV]. It has shown promising immunogenicity and protective efficacy in SPF chickens [12].

Reports of IBV outbreaks in vaccinated flocks raise concerns about possible genetic divergence between vaccine strains and currently circulating field isolates. Phylogenetic analysis of the S1 gene, which encodes the spike glycoprotein responsible for virus attachment and host immunity interaction, is widely accepted as a reliable approach for IBV genotyping and vaccine–field strain comparison [13]. By identifying the genetic distances between vaccine and field strains, researchers can assess the likelihood of effective cross-protection and update vaccination strategies accordingly.

This study aims to characterize and compare the S1 gene sequences of four 793/B-like vaccine strains used in Iran [including three commercial vaccines and one domestic strain developed by the Razi Vaccine and Serum Research Institute] with earlier 793/B-like field isolates. The findings will provide essential insights into the genetic relationships among these strains and support improved vaccine selection and IBV control measures in Iranian poultry.

2. Materials and Methods

2.1. Sample Collection and Virus Propagation

Four 793/B-like infectious bronchitis virus [IBV] vaccine strains were selected for sequencing and phylogenetic analysis. They included three commercial vaccines: POLIMUN IB MULTI BK-07 strain [BioTestLab]; Avishield IB GI-13 strain [Dechra]; AVIVAC-IB A/91 strain [AVIVAC] produced by international companies. The fourth vaccine strain analyzed in this study, 793/B.08IR, was developed and produced locally by the Razi Vaccine and Serum Research Institute, a national institution in Iran.

2.2. RNA Extraction and cDNA Synthesis

Viral RNA was extracted from the vaccine vial using a commercial RNA extraction kit [CinnaGen, Iran] following the manufacturer's protocol. The purity and concentration of the RNA extracted were identified by a NanoDrop spectrophotometer. The synthesis of complementary DNA [cDNA] was performed by a reverse transcription kit [CinnaGen, Iran] using random primers for amplification of the viral genome.

2.3. Polymerase Chain Reaction [PCR] and Sequencing

To amplify the full S1 gene of IBV vaccine strains, we used a previously validated primer set targeting the entire coding region of the S1 subunit around 1620 bp. The primers were: Forward Primer [S1-F]: 5'-TGA AAA CTG AAC AAA AGA C-3' and Reverse Primer [S1-R]: 5'-CAG ATT GCT TACA ACC ACC-3'[13]. PCR was performed under optimized conditions, and amplicons were visualized by gel electrophoresis using 1.5% agarose gel stained with ethidium bromide. Positive PCR products were purified and submitted for Sanger sequencing [Codon Genetic Group, Iran].

2.4. Phylogenetic Analysis

The generated sequences were edited and assembled using BioEdit software, and multiple sequence alignments were performed using Clustal W. The phylogenetic relationships were resolved using the maximum likelihood method in MEGA software with 1,000 bootstrap replicates. The vaccine strains that were newly sequenced were compared with the earlier characterized 793/B-like IBV Iranian and other country isolates to identify their genetic relatedness and potential antigenic divergence. Genetic distances among vaccine strains and Iranian circulating IBV isolates were compared for determining their cross-protective efficacy potential. GenBank reference sequences were included to facilitate broader comparative analysis. The findings were interpreted in relation to vaccine selection and field performance.

3. Results

The four vaccine strains were compared with a panel of previously characterized Iranian 793/B-like field isolates and reference strains retrieved from GenBank, specifically : 4/91 [MSD-AF093793] Classic 793/B reference strain; IB88 [Iranian variant] Genetically close to 793/B.08IR; 1/96 [CEVA-MK680010] European 793/B-like strain; Multiple archived Iranian field isolates [Previously sequenced and stored in national and GenBank databases] including: IR/773/2001; IR/794/2002. In total, 11 sequences were analyzed in this study. These included: 4 vaccine strains newly sequenced; 3 reference strains retrieved from GenBank (mentioned above); 4 previously characterized Iranian 793/B-like field isolates, selected from archived national datasets and GenBank entries, representing diverse geographic and temporal origins.

Pairwise sequence comparisons based on the S1 gene revealed high nucleotide identity among the evaluated 793/B-like IBV vaccine strains. The BK-07 [BioTestLab], GI-13 [Dechra], and A/91

[AVIVAC] vaccines showed 100% identity with each other, indicating they are virtually identical at the S1 gene level [Table 1].

Table 1. Genetic similarity of 793/B-like IBV Vaccine Strains Based on S1 Gene Phylogeny

		1	2	3	4	5	6
1	4/91 (MSD-AF093793)						
2	IB88 Vaccine (IR)	98					
3	1/96 (CEVA-MK680010)	95	92				
4	BK-07 (BioTestLab)	100	98	95			
5	GI-13 (Dechra)	100	98	95	100		
6	A/91(AVIVAC)	100	98	94	100	100	
7	793/B.08IR (Razi)	98	100	92	98	98	98

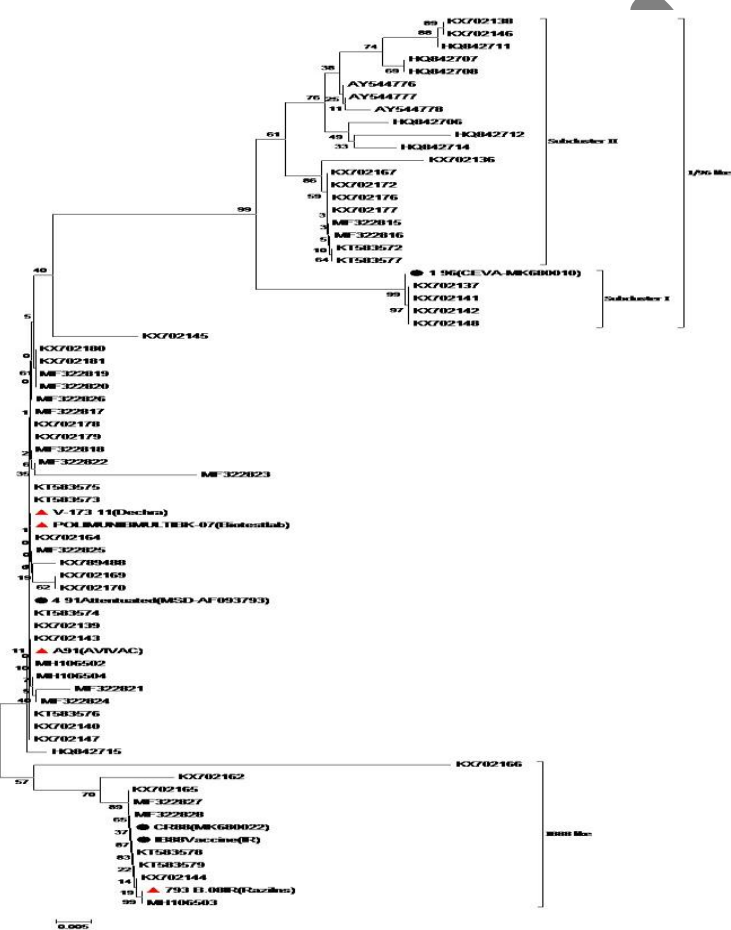


Figure 1. Phylogenetic tree of four 793/B-like vaccine strains used in Iran with earlier 793/B-like field isolates. Phylogenetic analysis was performed with MEGA 7 software, using Maximum

Likelihood method based on General Time Reversible model with 1000 bootstrap. Red triangles represent for vaccine strains in this study. Black circles represent for 793/B IBV vaccine strains.

The Razi Institute strain [793/B.08IR] exhibited 98% identity with the three commercial vaccines. Similarly, it showed 100% identity with the IB88 vaccine and 98% identity with the 4/91 strain, suggesting a strong genetic relationship with both locally used and internationally recognized vaccines [Fig 1]. Three commercial vaccines [POLIMUN, Avishield and AVIVAC] shared 100% identity with the classic 4/91 strain [MSD-AF093793] and 98% identity with both IB88 vaccine [IR] and 793/B.08IR [Table 2]. The CEVA 1/96 strain [MK680010], included as a reference genotype, showed 95% identity with the commercial vaccines and 92% with the Razi strain, supporting its classification within the broader 793/B-like group [Fig 2]. Importantly, all vaccine strains demonstrated high nucleotide identity (92–100%) with previously sequenced Iranian 793/B-like field isolates, including IR/773/2001 and IR/794/2002, confirming their genetic proximity and continued relevance for protecting against circulating IBV variants in Iran.

Table 2. Genetic Clustering of 793/B-like IBV Vaccine Strains Based on S1 Gene Phylogeny

Vaccine Strain	Clustered Group
A/91 (AVIVAC)	4/91-like
GI-13 (Dechra)	4/91-like
BK-07 (BioTestLab)	4/91-like
793 B.08IR (Razi)	IB88-like

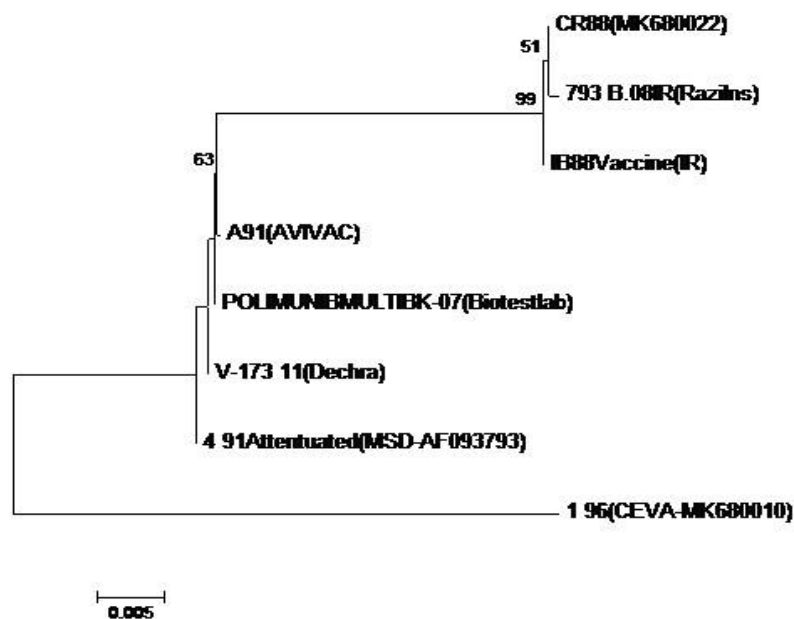


Figure 2. Phylogenetic tree for clustering of 793/B-like IBV vaccine strains based on S1 gene phylogeny

Discussion

The findings of current research demonstrate a high degree of genetic similarity among 793/B-like vaccine strains and relatedness to earlier 793/B-like field isolates circulating in Iranian poultry, supporting their continued relevance for IBV control in the region.

The 793/B genotype, originally described in Europe in the early 1990s, has since become one of the most widely used backbones for IBV vaccines due to its broad antigenic coverage and capacity to confer cross-protection against various field strains [1,14]. In Iran, the 793/B-like strains have been endemic for over a decade, with multiple studies confirming their dominance in both broiler and breeder flocks [9,10]. However, ongoing IBV outbreaks despite widespread vaccination have raised concerns regarding vaccine efficacy, often attributed to genetic drift or recombination events leading to antigenic variation in circulating strains [7,8]. Despite these close relationships, even

142 minor variations in the S1 gene—especially within the hypervariable regions—can result in
143 significant antigenic differences, potentially reducing vaccine efficacy [8]. Field reports of IBV
144 infection in vaccinated flocks may thus be explained by subtle mismatches or co-circulation of
145 other genotypes [e.g., QX, Variant 2] not covered by 793/B-like vaccines [15,16]. Moreover,
146 immunological interference between different vaccine serotypes administered simultaneously or
147 in sequence can also influence protective outcomes [17].



148 Phylogenetic grouping in this study confirmed that the commercial vaccines cluster within the
149 4/91-like group, while the Iranian Razi strain [793/B.08IR] aligned more closely with IB88-like
150 variants. 793/B.08IR is a live attenuated vaccine tailored to the Iranian 793/B field isolates to
151 improve protection where imported vaccines showed limited efficacy [12]. Although both [4/91
152 and IB88] belong to the broader 793/B serotype, such subgrouping may have practical implications
153 for vaccine selection and field performance. Previous research has shown that heterologous prime-
154 boost strategies [e.g., combining Mass and 793/B types] can enhance protection compared to
155 monovalent regimens, particularly when the field virus diverges antigenically from the vaccine
156 strain [18,19]. Therefore, continued molecular surveillance is essential to detect genetic shifts in
157 circulating IBV strains and assess vaccine–field strain compatibility. Incorporating whole-genome
158 sequencing and serological studies alongside S1 gene phylogeny would provide a more
159 comprehensive understanding of vaccine effectiveness under field conditions.

160 While it is true that live attenuated IBV vaccines can replicate and be transiently shed post-
161 vaccination, we do not believe that the field isolates analyzed in this study represent circulating
162 vaccine viruses for the following reasons: firstly, the field isolates used for comparison were
163 collected from multiple outbreaks across different provinces and time periods, often weeks to
164 months after vaccination. These isolates were obtained from flocks showing clinical signs of IBV

infection, not from routine post-vaccination sampling. Secondly, although high sequence identity was observed between vaccine strains and field isolates, minor but consistent nucleotide differences were present in the hypervariable regions (HVRs) of the S1 gene. These differences suggest natural evolution or recombination, rather than direct shedding of vaccine strains. Thirdly, vaccine strains clustered tightly within the 4/91-like or IB88-like groups, whereas some field isolates showed slight divergence, forming subclusters that are not identical to any vaccine strain. This pattern supports the idea that field strains are genetically related but not identical to vaccine strains. Finally, previous studies in Iran and neighboring countries have reported co-circulation of multiple 793/B-like variants, some of which differ subtly from vaccine strains despite shared ancestry. These findings reinforce the need for ongoing molecular surveillance to distinguish vaccine-derived sequences from endemic field strains. While transient detection of vaccine virus post-administration is possible, the isolates used in our study were selected based on clinical relevance, genetic divergence, and epidemiological context. Therefore, we conclude that they represent circulating field strains, not residual vaccine viruses.

In conclusion, the high sequence identity observed in this study supports the ongoing use of current 793/B-like vaccines in Iran, including the domestic Razi formulation. However, proactive monitoring and periodic reevaluation of vaccine strains remain critical to ensure sustained protection and minimize IBV-related economic losses in the poultry industry.

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Authors' Contribution

Study concept and design: A.G, H.H.

187 Analysis and interpretation of data: A.G, Z.ZK.

188 Drafting of the manuscript: F.J, A.B, S.S.

189 Acquisition of Data: A.S, A.R, M.M,

190 Critical revision of the manuscript for important intellectual content: R.M, H.H.

191 Study Supervision: A.G.

192 Contributed to the literature review and writing the manuscript; E.K, G.D, N.M.

193 Provided critical revisions to the manuscript and contributed to writing the manuscript; R.M, N.M

194 **Ethics**

195 Not Applicable.

196 **Conflict of Interest**

197 The authors declare that they have no conflicts of interest to disclose.

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200 **Data Availability**

201 The data that support the findings of this study are available on request from the corresponding
202 author.

203 **Reference**

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