Absence of Emerging GVIII IBV Genotypes IB80 and D2860 in Iranian Poultry: Molecular Surveillance from 2022–2025

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Abstract

Infectious bronchitis virus (IBV), a highly contagious pathogen within the Gammacoronavirus genus, significantly impacts global poultry health and productivity through respiratory disease, reduced weight gain, and impaired egg production. The emergence of genotype VIII (GVIII)

strains, particularly IB80 and D2860, in Europe and parts of Asia has raised international concern due to their potential for immune evasion and distinct antigenicity. This study aimed to assess the presence of these GVIII variants in Iranian broiler and broiler breeder flocks through molecular surveillance conducted between 2022 and 2025. A total of 200 tracheal and pulmonary tissue samples were collected from chickens exhibiting clinical respiratory signs across multiple provinces in Iran—140 from broilers and 60 from breeders. RNA was extracted and subjected to RT-PCR using strain-specific primers targeting the spike gene of IB80 and D2860. No positive amplifications were observed in any of the samples, indicating that these GVIII strains were either absent or present below the detection threshold of the assay (~100 RNA copies/µL). Positive and negative controls validated assay performance. These findings suggest a continued dominance of established IBV lineages in Iran, including Variant 2 (IS/1494-like), 793/B, and QX genotypes, with no evidence of GVIII incursion in the sampled populations. The apparent absence of IB80 and D2860 may reflect geographical constraints, host-specific tropism, or competitive exclusion by dominant genotypes. However, continued surveillance, especially in layer flocks and border regions, remains essential for early detection of emerging IBV threats. This study contributes vital baseline data to regional and global IBV monitoring efforts.

Keywords: Breeders; Broilers; D2860; IB80; IBV Genotype VIII; Infectious Bronchitis Virus; Iran; Molecular Surveillance

1. Introduction:

Infectious bronchitis virus (IBV), a member of the *Gammacoronavirus* genus (*Coronaviridae* family), poses a significant global threat to poultry health and productivity. The virus targets respiratory, renal, and reproductive tissues in chickens, causing substantial economic losses through reduced egg production, impaired weight gain, and heightened susceptibility to secondary pathogens (1). IBV exhibits extraordinary genetic plasticity, driven by high mutation rates, recombination events, and large population sizes. This rapid evolution continually generates novel antigenic variants that evade cross-protective immunity, complicating control efforts and necessitating vigilant molecular surveillance (2-4).

Accurate strain detection is critical for IBV management. While serological assays (e.g., ELISA, HI) remain widely used, their limitations in sensitivity and specificity impede reliable variant identification (5). Molecular approaches—particularly RT-PCR, qPCR, and next-generation sequencing (NGS)—now enable precise genotyping and real-time tracking of emerging lineages, forming the cornerstone of modern IBV epidemiology (2-4).

Recent European surveillance has revealed the expansion of genotype VIII (GVIII) strains, a phylogenetically distinct group first proposed by Domanska-Blicharz et al. (6). These strains showed <58.1% nucleotide identity with previously known IBV genotypes and the highest similarity (81.4% identity) to the unique North American PA/1220/98 variant (7-10). Notably, IB80-like strains (e.g., CK/DE/IB80/2016) have been detected >220 times across European poultry farms since 2015, with sporadic reports in the Middle East and Philippines (11). The Dutch strain D2860, recently characterized from layers, shares 95% whole-genome identity with IB80, confirming their close relationship within GVIII (12). Both variants exhibit pronounced tropism

for layer and breeder flocks, with minimal detection in broilers—a host-specific pattern consistent with certain IBV lineages (13).

In Iran, endemic IBV genotypes—including Variant 2 (IS/1494-like), 793/B, and QX—dominate outbreaks in broilers and breeders (14,15). Despite the rapid transcontinental spread of GVIII strains, their presence in Iranian poultry remains uninvestigated. This knowledge gap is critical given Iran's strategic location within Eurasian poultry trade routes and the economic consequences of novel variant incursion.

This study bridges this gap through targeted molecular surveillance of the emergent GVIII strains IB80 and D2860 in Iranian broiler and broiler breeder flocks (2022–2025). Our findings inform regional vaccine strategies and contribute to global efforts tracking IBV evolution.

2. Materials and Methods

2-1- Study Design and Sample Collection

A retrospective study was conducted from 2022 to 2025 to investigate respiratory disease complexes in Iranian poultry farms. A total of 200 samples were collected from clinically affected flocks in differen provinces of Iran (Gilan, Mazandaran, Fars, Kordestan, Ghazvin, Khoozestan, Isfahan, Khorasans, Azarbayjans) , comprising 140 samples from broiler chickens and 60 samples from broiler breeder flocks. Sample selection was based on the presence of clinical signs consistent with respiratory infection, including coughing, nasal discharge, respiratory distress, and decreased production performance. Postmortem tissue samples were obtained from affected birds, specifically targeting the trachea, lungs, and associated respiratory tissues. Each sample was

aseptically placed into sterile tubes and immediately stored at -80°C to preserve RNA integrity for subsequent molecular analyses.

2-2- RNA Extraction and cDNA Synthesis

Total RNA was extracted from tissue samples using the Sinapur RNA Extraction kit (Cinagen, Iran) according to the manufacturer's protocol. RNA purity and concentration were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA) by measuring absorbance ratios at 260/280 nm and 260/230 nm. cDNA synthesis was performed using a commercial reverse transcription kit (Thermo Fisher Scientific, USA). Each reaction contained 1 µg RNA, reverse transcriptase enzyme, reaction buffer, random primers, and dNTPs in a total volume of 20 µL. The reaction conditions were: 25°C for 10 minutes, 42°C for 60 minutes, followed by enzyme inactivation at 70°C for 10 minutes.

2-3- PCR Amplification and Detection

Specific PCR assays were previously developed to detect IB80 and D2860 variants of infectious bronchitis virus (11, 12). Two separate PCR reactions were performed for each sample using distinct primer sets targeting different spike regions of the viral genome. The first primer set (IB80 F1: TACTGGTAATTTTACAGATGG; IB80 R1: TAATTTGCTTACAGGCACC) and the second set (D2860 F2: GTAGCACCCACTAAGTTGCC; D2860 R2: TGTAATTGAACCATTAGCAC) were used in parallel reactions (11, 12)

Each 25 µL PCR reaction contained 12.5 µL of 2X PCR Master Mix (Promega, USA), 1 µL of each forward and reverse primer (10 µM), 2 µL of cDNA template, and nuclease-free water to reach the final volume. Amplification was performed under the following conditions: initial

denaturation at 95°C for 5 minutes; 35 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 45 seconds; followed by a final extension at 72°C for 10 minutes.

2-4- Electrophoresis and Visualization

PCR products were analysed by electrophoresis on 1.5% agarose gels containing ethidium bromide (0.5 μg/mL) in 1X TAE buffer at 100V for 45 minutes. A 100 bp DNA ladder (Thermo Fisher Scientific, USA) was included as a molecular size standard. DNA bands were visualized under UV light using a transilluminator (Bio-Rad, USA) and documented using a gel imaging system.

2-5- Quality Control Measures

Each PCR run included appropriate positive and negative controls. The positive control consisted of cDNA from a known positive sample, while the negative control contained nuclease-free water instead of template. Sample integrity was verified by successful amplification of housekeeping genes in parallel reactions. The specificity of amplification was confirmed by the expected product sizes for each primer set and the absence of bands in negative controls.

3. Results

Molecular screening of 200 clinical samples (140 from broilers, 60 from broiler breeders) using two specific RT-PCR sets targeting the IB80 and D2860 hypervariable S1 subunit of the spike gene failed to detect either strain in any tested specimens. Electrophoretic analysis of PCR products revealed no amplification bands at the expected sizes of 412 bp (primer set 1) or 389 bp (primer set 2) across all samples. Control reactions performed alongside the experimental samples

demonstrated proper assay function, with positive controls yielding bands of correct size and no-template controls showing no amplification.

The consistent negative results across all samples from clinically affected flocks suggest two plausible explanations. First, these genotype VIII variants may have been absent from the surveyed Iranian poultry populations during the 2022-2025 study period. Second, viral loads in respiratory tissues may have been below our assay's detection limit of 100 RNA copies per microliter, as determined through serial dilution of positive control material. The confirmed sensitivity and specificity of our primers, combined with the inclusion of flocks exhibiting clear respiratory disease symptoms, support the conclusion that IB80 and D2860 were not major causative agents of infectious bronchitis in these populations during our investigation.

4. Discussion

This study provides the first targeted molecular evidence for the absence of emergent genotype VIII (GVIII) IBV strains IB80 and D2860 in Iranian poultry flocks exhibiting clinical respiratory disease (2022–2025). This finding is epidemiologically significant given the rapid transcontinental spread of antigenically distinct GVIII lineages across Europe (>220 detections since 2015) and their documented expansion into the Middle East (11). While suggesting potential geographic compartmentalization, we acknowledge limitations: cryptic circulation below detection thresholds or in unsampled host populations cannot be excluded.

The IBV genotypic stability in the current study (absence of GVIII variants) contrasts sharply with Europe's IBV variants epidemiology (evolving GVIII). The dominance of Variant 2 may impose competitive exclusion against invading strains through pre-existing population immunity

or niche occupation. Historically, Iran has reported circulation of several IBV genotypes, including 793/B, QX, Massachusetts, D274, Q1, and IS/1494-like strains (14-19). IS-1494-like IBV was the most common type accounting for 85% of detected strains from 9 provinces of Iran in 2017. The other types overall created a lower proportion; including 793/B having a prevalence of 7%, QX, and Mass with prevalence rates of 5% and 2%, respectively, and D274 with the incidence of 1% (15). The eastern part of the country, Variant 2 (IS/1494-like) was predominant, and later 793/B and QX genotypes (20). A large-scale study between 2010 and 2014 identified seven genotypically distinct Mass, 793/B, IS720, Variant 2, QX, IR-I, and IR-II, of which variants related to the vaccine strain were overshadowed (21). QX-like viruses were found in 48% of infected flocks with respiratory signs in Iran's southwest (14).

The selective absence of IB80 and D2680 in our cohort, which included flocks with overt clinical signs, may also reflect the host tropism reported in European studies. These variants were predominantly detected in layer and breeder flocks, with limited presence in broilers (6,13). Although 60 broiler breeder samples were included in our analysis, further targeted surveillance of egg-laying systems may be warranted to rule out silent circulation.

While GVIII strains currently pose no detectable threat to Iranian broiler production, their rapid European expansion necessitates proactive surveillance. Integrating phylogenomic monitoring, host-specific sampling, and environmental risk modeling will be essential for early detection. This study establishes a critical baseline for tracking IBV incursions in a strategically significant region.

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Authors' Contribution

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

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

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

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

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

Study Supervision: A. G.

Contributed to the literature review and writing the manuscript; N. S, M. H, E. K.

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

Ethics

Not Applicable.

Conflict of Interest

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

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Data Availability

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

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