INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY ISSN Print: 1560–8530; ISSN Online: 1814–9596 24–0030/2024/31–6–465–473 DOI: 10.17957/IJAB/15.2165 http://www.fspublishers.org

**Review** Article



# A Comprehensive Review on Epidemiological Insights of Infectious Bronchitis Virus in South Asian Region

Bilal Javid<sup>\*</sup>, Tahira Kamal, Farhana Amin, Muhammad Naeem Riaz and Hafiz Muhammad Bilal Akhtar

National Institute for Genomics and Advanced Biotechnology, NARC, Islamabad, Pakistan \*For correspondence: bilaljavid824@gmail.com

Received 17 January 2024; Accepted 19 March 2024; Published 16 April 2024\_\_\_\_\_

# Abstract

Infectious bronchitis virus (IBV) is a highly mutating virus that affects both vaccinated and unprotected chicken flocks and causes enormous economic losses worldwide, so it is very critical to gain a deeper understanding of this virus. It is classified as gamma corona virus which belongs to family of coronaviridae. It leads to infectious bronchitis in poultry which is a contagious disease. The upper respiratory tract and reproductive tract are mostly affected by this disease. There are many strains which have been identified globally and cause nephritis along with other complications. The occurrence of different strains is the result of recombination and mutation in the viral genome, which makes it very difficult to identify and control. This virus contains three major structural proteins that are encoded by the virus, one of which is the highly variable spike (S) glycoprotein. The S1 portion surface protein of IBV virus is involved in hem-agglutination and pathogenicity due to the presence of virus neutralizing epitopes. The amplification trough RT-PCR and S1 glycoprotein sequence analysis made the diagnosis of Infectious bronchitis virus possible. The phylogenetic analysis of different strains of S1 gene helps in identifying the similarity index of this virus with other related virus strains. The objective of current review is to deliver an overview of the IBV variants or strains those are currently in circulation in commercial poultry in South Asian region and it focuses on the point that particular vaccine should be prepared according to the prevailing local strain of particular area. In order to develop effective vaccine, vaccine matching is very crucial process because of the rapidly changing nature of infectious bronchitis virus. The details about IBV types provided here are taken from published articles and Submissions at Gene Bank. The phylogenetic analysis was conducted to check the relationship between different IBV strains in some South-Asian countries. © 2024 Friends Science Publishers

Keywords: IBV; Genetic type; S1 gene; South Asia; Vaccine matching; Phylogenetic analysis

# Introduction

One of the most economically significant diseases of poultry is infectious bronchitis virus (Uddin et al. 2016). This virus is a continuously evolving with an envelope that carries a positive-sense, single-stranded RNA genome. It is classified within the Gamma coronavirus genus of the Coronaviridae family. The poultry industry worldwide has faced remarkable economic losses due to IBV-accompanying conditions such as tracheitis, proventriculitis, nephritis, salpingitis and significant decrease in egg production (Zhang et al. 2020). The important structural proteins are encoded by the IBV genome which are N protein (nucleocapsid), M (membrane-protein), S (spike-glycoprotein) and E (small membrane-protein). IBV can cause harm to the host's kidneys, reproductive system, and respiratory tract by replicating within the epithelium of numerous organs (Wit et al. 2019). IBV strains are categorized into 35 lineages having seven genotypes using a novel classification technique (GI to GVII) (Valastro et al. 2016; Chen et al. 2017; Jiang et al. 2017; Ma et al. 2019). It is typical to see the disease in vaccinated chickens also, which has a significant negative economic impact globally (Sumi et al. 2012). Respiratory diseases such as tracheal rales, coughing, and sneezing, as well as excessive mucus formation and accumulation in the bronchi, decreased broiler growth, nephritis, urolithiasis, and permanent oviduct damage, which results in high mortality rates and abnormal egg production (Worthington et al. 2008; Bickerton et al. 2018). The size of IBV viral genome is approximately 27.6 kb having 5 prime and 3 prime noncoding regions (Abro et al. 2012a). It contains three most important structural proteins that are encoded by the virus, one of which is the highly variable spike (S) glycoprotein, which is interpreted as a pre-protein or protein prior to being fragmented into the N-terminal (S1) and the C-terminal (S2) glycol-polypeptides (Farsang et al. 2002). The portion of surface protein (S1) of IBV virus is involved in hemagglutination and pathogenicity due to the presence of virus neutralizing epitopes (Abro et al. 2012b). The S1 protein's antigenic determinants alter, resulting in the creation of novel

To cite this paper: Javid B, T Kamal, F Amin, MN Riaz, HMB Akhtar (2024). A comprehensive review on epidemiological insights of infectious bronchitis virus in south Asian region. *Intl J Agric Biol* 31:465–473

strains and genotypes (Promkuntod et al. 2015). IBV serotypes differ in their S1 glycoprotein by around 20 to 25%. On the other hand, variations of up to 50% have been noted, which has an impact on cross-protection against virus strains that are developing or reoccurring (Ennaii et al. 2020). The S1 gene of IBV is most frequently addressed in molecular epidemiological investigations to describe (e.g., genotyping) and comprehend the spread of the virus because of its genetic diversity (Bande et al. 2017). It is possible to determine the path of the virus distribution and identify the strains of the virus that are most common in a given location by using sequencing and phylo-genomics (Zulperi et al. 2009). However, because IBV is an extremely fluctuating coronavirus, the current occurrence of novel strains significantly reduces the efficacy of IBV vaccines (Fan et al. 2018). The vaccines' low cross-protection rates unavoidably impede the disease's prevention and control (Jordan 2017). IBV can take many different forms that are difficult to manage. Their vaccines sometimes do have the ability of cross protection. Attenuated live vaccines are given to broilers and pullets, and killed vaccines are typically given to layers and breeders. Effective control requires the identification of the virus that causes the disease and the subsequent administration of an appropriate vaccine against it (Cavanagh and Naqi 2003). The emergence of new serotypes as a result of some changes in amino acid sequence is due to the immunological stress triggered by extensive use of vaccines. The other filed strains emerge due to the consequence of mixed infection or decline of most prevailing serotype (Liu et al. 2006). Numerous IBV serotypes have been identified globally, and there is minute to no crossimmunity between the various antigenic variants (Cavanagh 2007). This results in an increasing number of immunological failures and large financial losses for the chicken sector Chen et al. 2017).

# Molecular characterization of BV in India

A study was conducted in India to identify and characterize two strains of the infectious bronchitis virus (IBV) from field occurrences in broiler chickens in 2008 and 2010 which were India/NMK/72/IVRI/10, India/LKW/56/IVRI/08 and respectively. The two Indian IBV isolates presented 73% similarity among them according to nucleotide sequencing analysis, while India/LKW/56 and India/NMK/72 were 99% similar with the 4/91 (it is pathogenic strain in the UK), JP/Wakayama/2/2004 (Japan), and TA03 (China). The presence of 4/91 (793/B) IBV nephron-pathogenic variant existing in India was confirmed for the very first time. Phylogenetic Analysis revealed that the isolate India/LKW/56/IVRI/08 formed a group with THA280252 (Thailand), and the isolated strain, India/NMK/72/IVRI/10 formed a separate group with 4/91 pathogenic (UK) (Sumi et al. 2012). The furthermost prevalent IBV variants identified in India since 1991 were the (India/Mass/16-V-AD/07) in Mass genotype and 793B strain (Elankumaran et al. 1999).

The strain India/PDRC/Pune/9/99 and various nephropathogenic variants or strains of Infectious Bronchitis were recognized in 1991 (Bayry et al. 2005). The five IBV strains were identified and described as Anand isolates. These isolates were isolates in Guirat. India and were compared with already isolated field strains of IBV in Gujrat (Patel et al. 2015). These isolates with other strains are given in Table 1. Another study found that 20 field strains of IBV were examined in India between 2003 and 2011 using RT-PCR and sequencing of the S1 gene's HVR I and HVR II. The three isolates (I. IND-TN-168-06, II. IND-TN-280-10, III. IND-TN-290-11) out of 20 were totally new variants which matched with GI-24 lineage. The Fourteen isolates (a. IND-113-03, b. IND-114-03, c. IND-TN-04-03, d. IND-TN-20-03, e. IND-TN-92-03, f. IND-TN-95-03, g. IND-TN-97-03, h. IND-TN-98-03, i. IND-AP-151-05, j. IND-KA-152-05, k. IND-TN-162-06, 1. IND-TN-163-06, m. IND-TN-270-09, n. IND-TN-183-09) were grouped in GI-1 lineage. The further two (02) field isolates (I. IND-TN-174-07, II. IND-TN-175-07) grouped into the GI-13 genetic lineage (Raja et al. 2020). Some published IBV isolates of India with accession numbers are given in Table 1.

#### Molecular characterization of IBV in China

In 2019, HeN-1/China/2019, HeN-2/China/2019, and HeN-101/China/2019 were shown to be three extremely aggressive IBV strains in China. The recently found IBV strain was closely linked to the ck/China/I0529/17 strain and classified into the GI-19 genotype clade on the basis of genetic sequence and phylogenetic study of the full S1 gene, despite the fact that the gross pathological demonstration of two IB outbreaks was divergent. This work shed light on recently occurring IBV epidemics in poultry with IBV vaccinations and identified the genetic traits of three virulent GI-19 IBV strains, demonstrating the necessity of implementing appropriate preventative measures and management tactics. After Molecular detection, four IBV strains were isolated comprising HeN-101/China/2019, and HeN-102/China/2019, HeN-1/China/2019, HeN-2/China/2019. After complete sequencing the complete S1 gene Hen-1 and Hen-2 strains showed 99.9% similarity, while Hen-101 and Hen-102 showed 100 similarities exploring the fact that these two outbreaks were triggered by single IBV strain (Zhang et al. 2020). In 2018, ck/CH/LDL/150434--I (LDL/150434-I), ck/CH/LDL/150434-II (LDL/150434-II), and ck/CH/LDL/150434-III (LDL/150434-III) are the three distinct IBV genotypes/serotypes that were identified in chicken (Han et al. 2018). The Novel IBV strain, ck/CH/LGX/111119 was identified in 2017 and grouped in GI-28 lineage. This new novel strain may be the result of the recombination of IBVs in LX4 genotype and non-identifies IBV strain, or S1 gene of unidentified IBV or mutations in S1 gene of IBVs of LX4 genotype (Chen et al. 2017). Nine distinct genetic families, including Mass- and 793B-type

Country	Strain	Туре	Gene Bank No#	Reference
	India/ LKW/56/IVRI/08	Mass	HM163471	Sumi et al. (2012)
India	India/Mass/16-V-AD/07	Mass	HM179146	Elankumaran et al. (1999)
	India/NMK/72/ IVRI/10	4/91	HM748585	Sumi et al. (2012)
	India/PDRC/Pune/9/99	(Unique)	AY091551	Bayry et al. (2005)
	ANAND/GUJ/IBV1/2013	Cluster I	KJ577258	Patel et al. (2015)
	ANAND/GUJ/IBV2/2013		KJ577259	
	ANAND/GUJ/IBV3/2013		KJ577260	
	ANAND/GUJ/IBV4/2013		KJ577261	
	ANAND/GUJ/IBV5/2013		KJ577262	
	IND-TN-168-06	Variant IBV	JX966396	Raja et al. (2020)
	IND-TN-290-11	(GI-24)	JX966403	
	ND-113-03	(Mass 41 IBV)	JX966392	
	IND-114-03	GI-1	EF165593	
	IND-TN-04-03		EF165596	
	IND-TN-20-03		EF165597	
	IND-TN-92-03		EF165598	
	IND-TN-95-03		EF165599	
	IND-TN-97-03		EF165600	
	IND-TN-98-03		EF165601	
	IND-AP-151-05		JX966393	
	IND-KA-152-05		EF165595	
	IND-TN-162-06		JX966394	
	IND-TN-163-06		JX966395	
	IND-TN-270-09		JX966400	
	IND-TN-183-09		JX966399	
	IND-TN-174-07	UK 4/91 IBV	JX966397	
	IND-TN-175-07	(GI-13)	JX966398	
	IND-284-10	Indian nephron-pathogenic IBV	JX966402	

**Table 1:** Prevalence of different isolates of infectious bronchitis virus in India. The given isolates of IBV have different levels of similarity index of S1 gene sequence. This table contains the already published Indian IBV isolates with different Antigenic variations

viruses, reported in China: LX4, LDT3, LHLJ, BJ, LDL, N1/62, and LSC (Han et al. 2011). The wider distribution and high pathogenicity make the IBVs of LX4 and LDL groups more significant among all the identified genetic groups. The different strains were published with Gene Bank No. as China/LX4/QX/99 (AF193423), China/LDT3/03 (AY702975), China/LHLJ/95I (DQ167141), China/BJ/97 China/LDL/Q1/98 (AY31965), with (AF286302), China/N1/62/JAAS/04 China/LSC/99I (AY839140), (DO167147). China/793B/Sichuan/06 (GO844991). China/Mass-H120/ SDLY0612/06 (EU857816) and Taiwan/LDL/Q1-3374/05 (DQ402364) (Jackwood 2012). In 2019 and 2020, two isolates of IBV, designated CK/CH/TJ1904 and CK/CH/NP2011, from many poultry farms in the provinces of Tianjin and Fujian were recovered, respectively. The CK/CH/TJ1904 and CK/CH/NP2011 strains whole genome sequences have been added to Gene Bank under the accession numbers MW815494 and MW815495 (Sun et al. 2021). For the previous 20 years, the QX (GI-19) genotype has dominated the Chinese population. It was initially identified in Oingdao, China, in 1996 (Xu et al. 2018) (Zhao et al. 2016). China's Guangxi province reported the first isolation of a GVI-1 strain in 2007. The strain, dubbed TC07-2, differed significantly from six other key genotypes in terms of evolution (Li et al. 2010). The respiratory tract tropism observed in GVI-1 strains may be caused by extensive gene 3 and S recombination (Ren et al. 2019). The S1 glycoprotein gene nucleotide sequences of the avian infectious bronchitis virus (IBV) strains Gray and JMK

were identified and cross-referenced with previously published IBV sequences (Kwon and Jackwood 1995). The new IBV GDTS13 strain was evaluated for vaccine production, which was common and most prevalent in 2016-2017 in southern China, GVI-1 is now the most common IBV genotype. Some published IBV isolates of China with accession numbers are given in Table 2. The Phylogenetic study was conducted on the basis of whole S1 gene sequences among 16 strains and 156 reference strains. In this experiment, GI-1 includes the mentioned strains as reference like Mass 41 and H120 having accession numbers AY561711, FJ888351, respectively (Chen *et al.* 2021).

## Molecular characterization of IBV in Pakistan

A trail was conducted to examine clinical samples of IBV in Pakistan, 358 out of 905 samples were found to be positive, with serotype distributions of Mass strain (43%), 4/91strain (51%), and various IBV variants (5%), respectively. A variant Pak-973 was recovered from Broiler Breeder flock through molecular characterization. IBV isolate was given name as KX013102\_NARC/973\_Pakistan\_2015. The phylogenetic study presented 93% resemblance with KF360983\_23/B/2008\_India. After sequencing the Pak-973 isolate, a difference of almost 7% was noticed with the rest of the variants and serotypes. The 13 Amino acid substitutions also made pak-973 different from rest of the isolates. The amino acid mutations were found on the hyper variable region 1 and hyper variable region 2s, which made

Country	Strain	Туре	Gene Bank No#	Reference
	China/LX4/QX/99	LX4	AF193423	Jackwood (2012)
China	China/LDT3/03	LDT3	AY702975	
	China/LHLJ/95I	LHLJ	DQ167141	
	China/BJ/97	BJ	AY319651	
	China/LDL/Q1/98	LDL	AF286302	
	China/N1/62/JAAS/04	Subgroup 1	AY839140	
	China/LSC/99I	LSC	DQ167147	
	China/793B/Sichuan/06	793B	GQ844991	
	China/Mass-H120/ SDLY0612/06	Mass	EU857816	
	Taiwan/LDL/Q1-3374/05	N1/62	DQ402364	
	CK/CH/GD/GDJM1206	GVI-1	MN193597	Chen et al. (2021)
	CK/CH/GD/GDSB1214	GVI-1	MN193599	
	CK/CH/GD/BJSN17	GVI-1	MN193588	
	HeN-1/China/2019	G-19	MN055627	Zhang et al. (2020)
	HeN-2/China/2019	G-19	MN055628	-
	HeN-101/China/2019	G-19	MN635798	
	ck/CH/LDL/150434–I	Mass	KT736031	Han et al. (2018)
	ck/CH/LDL/150434-II	LDT3	KT736032	
	ck/CH/LDL/150434-III	TWI like	KX077987	
	ck/CH/LGX/111119	GI-28	KX640829	Chen et al. (2017)
	CK/CH/TJ1904	GV1	MW815494	Sun et al. (2021)
	CK/CH/NP2011	GV1	MW815495	
	DY07	Genotype -I	GQ265927	Li et al. (2010)
	MN07	GenotypeI	GQ265946	
	DY05	Genotype -I	GQ265928	
	ZX07	GenotypeI	GQ265949	
	LZ07	Genotype II	GQ265944	
	NN04	Genotype-II	GQ265951	
	HY06	Genotype -III	GQ265941	
	CQ04-2	GenotypeIII	GQ265953	
	TC07-2	Genotype -VI	GQ265948	
	Ck/CH/LSD/091003	QX like	HM194708	Ren et al. (2019)
	Ck/CH/LDL/091022	QX like	HM194640	
	Ck/CH/LJL/090330	QX Like	HM194674	
	CK/CH/GD/GDJM502	GI-19 lineage	MN193595	
	CK/CH/GD/GDSB1220	GI-19 lineage	MN193600	

**Table 2:** Prevalence of different isolates of infectious bronchitis virus in China. The given isolates of IBV have different levels of similarity index of S1 gene sequence. This table contains the already published Chinese IBV isolates with different Antigenic variations

the Pak-973 as new IBV strain. This study focused on the point that vaccine matching should be done before the selection of vaccine to control IB in commercial poultry (Rafique et al. 2018a). The majority of IBV isolates in Pakistan was grouped into GI-24 lineage and some were classified in GI-13. One isolate UAF-8 was placed in GI-1 lineage. This study evaluated the criteria using the 9 novel sequences for all Pakistani isolates of IBV that are currently available. The 8 sequences of IBV isolates out of 9 were grouped in GI-13 and one was placed in GI-24 lineage. The isolates from the liver, kidney, and respiratory tract are included in the GI-24 lineage and strains of GI-13 lineage are mostly linked to samples isolated from the reproductive and respiratory system (Saleem et al. 2024). The Pakistani IBV isolates from different areas are given in Table 1. Another study which was conducted in Pakistan revealed the unique IBV strain named as Pak-786. It showed link with GI-13 lineage that comprise the vaccine as well as highly pathogenic field strains. The study backs up the idea that a range of variants arise via random accidental mutation and genetic recombination, which may cause genetic drift, as a result of the widespread use of live IBV vaccination strains with different origins. The study's emerging strain of IBV

highlights the necessity of including these variants in killed vaccine form into the affected region's immunization program (Rafique *et al.* 2018b). The most common strain of the infectious bronchitis virus was determined to be M-41, which was detected in 100% of layer flocks and roughly 67% of broiler flocks, with a total combined incidence of 88% in all flocks examined (Ahmed *et al.* 2007). Some published IBV isolates of Pakistan with accession numbers are given in Table 3.

#### Molecular characterization of IBV in Iran

A unique study demonstrated that samples were collected from (40) forty IB infected flocks from 4 different provinces of Iran. The samples were inoculated in to 9–11 days old chicken eggs (embryonated). After collection of fluid and RT-PCR, results showed that Four (04) isolates (IBV-83, IBV-29, IBV-80, and IBV-56) had high similarity (98.56 to 99.59%) to 4/91 serotype which belongs to (Pakistani strain) GI-13 lineage. Following phylogenetic analysis (on comparison of Nucleotide sequence), three isolates (IBV-80, IBV-16, and IBV-17) were classified as belonging to the GI-19 lineage (QX-like viruses), as they had 98 to 99% of the

Country	Strain	Туре	Gene bank No.	Reference
Pakistan	NARC/973_Pakistan_2015	(GI-24)	KX013102	Rafique et al. (2018a)
	IBV17/QAU/Pakistan/Talagang/2017 lung/trachea		MH703657	• · · ·
	IBV/Ahad51 2018 Pakistan liver		MW464186	
	IBV/Ahad559 2019 Pakistan liver		MW464189	
	CK/PAK/UDL/MS-05/LHR/2020 Pakistan lung trachea kidney		OL763345	
	CK/PAK/UDL/MS-02/RWPD/2020 Pakistan lung trachea kidney		OL763342	
	CK/PAK/UDL/MS-03/MULT/2020 Pakistan lung trachea kidney		OL763343	
	CK/PAK/UDL/MS-06/LHR/2020 Pakistan lung trachea kidney		OL763346	
	CK/PAK/UDL/MS-04/MULT/2020 Pakistan lung trachea kidney		OL763344	
	CK/PAK/UDL/MS-01/ABTD/2020 Pakistan lung trachea kidney		OL763341	
	UAF-10 2020 Pakistan kidney		MW525215	
	UAF-9_2018 Pakistan lung		MW525214	
	IBV/Ahad 196 2018 Pakistan liver		MW464188	
	IBV/Ahad13 2018 Pakistan liver		MW464185	
	CK/PK/UVAS-GM-026-Lalamusa/2019 Pakistan		MK689242	
	IBV/Ahad4 2018 Pakistan liver		MW464184	
	IBV/Ahad3 2018 Pakistan liver		MW464183	
	IBV19/QAU/Pakistan/Rawalpindi/2017 Pakistan lung	(GI-13)	MH703659	
	IBV20/QAU/Pakistan/Rawalpindi/2017 Pakistan lung		MH703661	Saleem et al. (2024)
	IBV21/QAU/Pakistan/Rawalpindi/2017 Pakistan lung		MH703660	
	IBV18/QAU/Pakistan/Rawalpindi/2017 Pakistan lung		MH703858	
	IBVQ2/QAU/Pakistan/Rawalpindi/2017 Pakistan lung		MH703663	
	IBVQ1/QAU/Pakistan/Rawalpindi/2017 Pakistan lung		MH703662	
	IBV4/QAU/Pakistan/Rawalpindi/2017 Pakistan lung		MH703655	
	chicken/Pakistan/PATH-IX 2019 Pakistan tissue homogenate		MW856023	
	UAF-8 2020 Pakistan Liver	(GI-1)	MW525216	
	NARC/786 Pakistan 2013	(GI-13)	KU145467	Rafique et al. (2018b)

**Table 3:** Prevalence of different isolates of infectious bronchitis virus in Pakistan. The given isolates of IBV have different levels of similarity index of S1 gene sequence. This table contains the already published Pakistani IBV isolates with different Antigenic variations

similarities to Iran and Iraq origin QX-like viruses. Two isolates IBV-34 and IBV-106, which belong to the GI-23 lineage (variant-2) share a 95-97% resemblance with Iranian variants of the GI-23 lineage. In this Current research, IBV was identified from 30% of the 40 flocks situated in diverse areas of Iran. Among these isolates, the 793/B serotype emerged as the most prevalent, with QX-like, variant-2 and Massachusetts isolates following in respective order of occurrence. It's noteworthy that all four lineages were identified to be actively circulating within these 40 flocks. The dominant IBV genotypes identified in specific regions of Iran include the GI-13, GI-19, and GI-23 lineages (Ghorbiani et al. 2020). In 1994, there was a report on the initial isolation of the IBV in Iranian chicken flocks. A study revealed that IBV isolates in Iran showed linkages to six different genetic groups. Group I had 40 field isolates (34%) that were most similar to Var2 (IS/1494/06 strain), Group II with 793/B serotype, Group III with QX like strain, Group IV with IS1720 strain, group V with Mass strain and Group VI showed similarity with IR1 genetic group (Najafi et al. 2016). Another molecular level study was conducted on infectious bronchitis virus in Iran to investigate the prevailing strains. This study documented Iran's first-ever identification of Q1 infectious bronchitis virus genotype which was originated from China from the proventriculus part of layers chickens. The newly identified strains were Iran/O1/UT-PCR-N1/ 2019 and Iran/O1/UT-PCR-N2/ 2019 linked to Q1 genotype. After sequencing both positive samples, the sequences were given the accession codes MN841015 and MN841016 for Iran/Q1/UT-PCR-N1/2019 and Iran/Q1/UT-PCR-N2/2019, respectively (Ghalyanchilangeroudi *et al.* 2020). Some published IBV isolates of Iran with accession numbers are given in Table 4.

## Molecular characterization of IBV in Bangladesh

In this study, total 371 organ samples—the lungs, kidney, and trachea-were obtained from breeder, broiler, and layer chickens and placed in sterile zipper-lock bags., and Sonali breed (local) chickens that were exhibiting clinical respiratory signs from 9 sampling areas. After processing of swab solution (Homogenization, Suspension, Centrifugation and RNA Extraction), the next step was undergone RT-PCR. The S1 gene specified primer and probe were used in RT-PCR according to OIE guidelines as given in Anonymous (2004). Samples found to be highly positive for IBV using RT-PCR were inoculated into 9-day Specific pathogen free (SPF) chicken egg embryos according to OIE guideline and observed for dwarfing and curling. The next step was harvesting fluid, S1 gene sequence and phylogenetic analysis. Out of 371 samples, 65 (almost 17.5%) samples were positive for IBV in different areas of Bangladesh. The commercial layer was highly prevalent (42.2%) and broiler chicken showed lowest positive samples that were 16 out of 134 (11.9%). The prevalence of local breed Sonali and broiler breeder chicken was 17 and 14.9%, respectively. The results showed that Isolates of IBV were similar with a QX-like and Indian isolates (Bhuiyan et al. 2019). The total 5 isolates of IBV were found in Bangladesh which showed different level of similarity with

Country	Strain	Туре	Genebank No.	Reference
Iran	IBV-34	GI-23	MK850426	Ghorbiani et al. (2020)
	1BV-106	GI-23	MK850429	
	1BV-80	GI-13	MK850432	
	1BV-83	GI-13	MK850428	
	1BV-29	GI-13	MK850425	
	1BV-56	GI-13	MK850431	
	1BV-8	GI-19	MK850423	
	1BV-17	GI-19	MK850424	
	1BV-16	GI-19	MK850430	
	1BV-35	GI-1	MK850427	
	IS-1494/UTIVO-27/2014	IS/1494/06 like	KT583593	
	IS-1494/UTIVO-66/2014 IS-1494/UTIVO-99/2014	(Variant2 like)	KT583597	(Najfi et al. 2016)
	IS-1494/UTIVO-90/2014		KT583598	
	IS-1494/UTIVO-97/2014 IS-1494/UTIVO-93/2014		KT583599	
	Iran/Variant 2/H840/14		KT583600	
	IS-720/UTIVO-15/2014		KT5835601	
	IS-720/UTIVO-114/2014		KP310028	
	IS-720/UTIVO-113/2014	IS720	KT583583	
	IR-Razi-HKM3-2010	like	KT283585	
	IR-Razi-HKM2-2010		KT583584	
	IR-I/H600/13		JN600612	
	IR-1/UTIVO-41/2015	IR-1 like (Iran-	JN600611	
	IR-1/UTIVO-117/2015	strains cluster)	KP310035	
	Iran/793B/UTIVO-1/2014		KT583580	
	Iran/793B/UTIVO-86/2014		KT583581	
	Iran/793B/UTIVO-48/2014	4/91 like	KT583572	
	Iran/793B/UTIVO-18/2014	793B like	KT583577	
	Iran/793B/UTIVO-108/2014		KT583576	
	IR-1062-GA		KT583573	
	IR-Razi-HKM4-2010		KT583579	
	Iran/QX/UTIVO-103/2015		AY544777	
	Iran/QX/UTIVO-6/2015		JN600613	
	Iran/QX/UTIVO-2/2015	QX like	KT583571	
	PCRLab/06/2012	-	KT583568 KT583567	
	Iran/Mass/UTIVO-111/2015		JX477827	
	Iran/Mass/UTIVO-22/2015		KT583566	
	Iran/Mass/UTIVO-46/2015	Mass like	KT583564	
	Iran/Mass/H650/13		KT583565	
	Iran/Q1/UT-PCR-N1/2019	Q1 Genetype	KP310053	
	Iran/Q1/UT-PCR-N2/2019	Q1 Genotype	MN841015 MN841016	Ghalyanchilangeroudi et al. (2020)

**Table 4:** Prevalence of different isolates of infectious bronchitis virus in Iran. The given isolates of IBV have different levels of similarity index of S1 gene sequence. This table contains the already published Iranian IBV isolates with different Antigenic variations

each other and hence these isolates were grouped after phylogenetic analysis. The two isolates of IBV were grouped in 4/91 type and two more isolates were grouped in Mass genotype. The remaining one was grouped in QX like genotype (Parvin *et al.* 2021). The IBV isolates are given with some other Bangladesh isolates in Table 5.

#### Molecular characterization of IBV in Afghanistan

According to first report on IBV in Afghanistan, between 2016 and 2017, IBV strains were found to be clustered into two different genotypes, LX4 (GI-19) and IS-1494 similar (GI-23) (34/45), according to phylogenetic analysis of all positive samples. The LX4 IBV (Afghan) is different from Iranian QX IBV. The Iraqi IBV (QX) showed almost 98.9% similarity with China QX like IBV stains. Regarding similarity to circulating strains in Iran and Iraq, IS-1494 had a 20% spread. It should be noted that frequent strains found in the area, such as Massachusetts and 793/B, were not found in this investigation. The study aimed to evaluate the percentage homology of partial sequences of nucleotide of

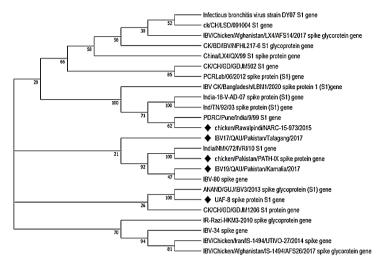
the S1 genes of certain Afghan IBVs. The obtained nucleotide and sequences of amino acids can be found in the Gene Bank with accession numbers MF322853-MF322867 (Sadri et al. 2019). It is evident that there are many different IBV variations on the globe today. The challenges lie in classifying these variants and connecting the results to the most effective vaccination plan for protection. However, genotyping offers convenience and speed, the present review highlights requirement for a standardized way of performing genotyping because different groups use different parts such as the S1 region of the S gene for strain comparisons, which makes the interpretation of outcomes very difficult. Another important thing to keep in mind is that the level of crossprotection in a chicken is determined solely by the vaccination, not by genetic or antigenic variations as determined by genotyping or serotyping (Wit et al. 2011). Cross-protection studies indicate that flocks can be protected against IS-1494 and QX by using heterologous vaccines with different genotypes, such as Massachusetts and 4/91 (Habibi et al. 2017). Some published IBV isolates of Afghanistan with accession numbers are given in Table 6.

**Table 5:** Prevalence of different isolates of infectious bronchitis virus in Bangladesh. The given isolates of IBV have different levels of similarity index of S1 gene sequence. This table contains the already published Bangladeshi IBV isolates with different Antigenic variations

Country	Strain	Туре	Gene Bank No#	Reference
	CK/ BD/IBV/NPHL1	QX like	MH631011.1	Bhuiyan et al. (2019)
Bangladesh	CK/BD/IBV/NPHL2	4/91 like	MH688060.1	
-	CK/ BD/IBV/NPHL3	4/914/91 like	MH685740.1	
	IBV_CK/Bangladesh/LBM1/2020-HVR1-2 S1	4/91 like	MW971986.1	Parvin et al. (2021)
	IBV_CK/Bangladesh/LBM5/2020-HVR1-2 S1	Mass like	MW971987.1	
	IBV_CK/Bangladesh/LT46/2020-HVR1-2 S1	Mass like	MW971994.1	
	IBV_CK/Bangladesh/LT1/2020-HVR1-2 S1	QX like	MW971988.1	
	IBV_CK/Bangladesh/LT57/2020-HVR1-2 S1		MW971990.1	

Table 6: Prevalence of different isolates of infectious bronchitis virus in Afghanistan. The given isolates of IBV have different levels of similarity index of S1 gene sequence. This table contains the already published Afghani IBV isolates with different Antigenic variations

Country	Strain	Туре	Gene bank No.	Reference
Afghanistan	Afghanistan/AFS21/2017	LX4 Like (GI-19)	MF322865	Sadri et al. (2019)
	Afghanistan/AFS14/2017	IS-1494 Like (GI-23)	MF322866	
	Afghanistan/AFS20/2017		MF322862	
	Afghanistan/AFS26/2017		MF322854	
	Afghanistan/AFS29/2017		MF322853	
	Afghanistan_AFS5_2017	LX4 Like	MF322867	
	IBV/Chicken/Afghanistan//AFS1/2016		MF322856	
	IBV/Chicken/Afghanistan//AFS7/2016		MF322857	
	IBV/Chicken/Afghanistan//AFS9/2016		MF322858	
	IBV/Chicken/Afghanistan//AFS15/2016		MF322859	
	IBV/Chicken/Afghanistan//AFS18/2016		MF322860	
	IBV/Chicken/Afghanistan//AFS19/2016		MF322861	
	IBV/Chicken/Afghanistan//AFS22/2016		MF322863	
	IBV/Chicken/Afghanistan//AFS23/2016		MF322864	



**Fig. 1:** Phylogenetic tree showing Antigenic diversity of the S1 gene for IBV strains generated using neighbor-joining 100 bootstrap replicates. The phylogenetic tree was constructed by using MEGA 11 software. Ten (05) Pakistani IBV strains were compared with IBV strains in selected countries of South-Asia. The Pakistani strains in this study were indicated by black arrows, The Fig. 1 contains the already published IBV isolates with different Antigenic variations in South-Asian region

#### **Control strategies**

The first step to control this economically significant disease in poultry requires identification of novel variant of IBV, which is linked to the disease outbreak in vaccinated birds. It is the need of an hour to develop a vaccine in order to control the newly circulating strain of IBV in particular area, because already available vaccines are unable to offer sufficient protection. The killed vaccines or attenuated live vaccines are usually developed for Novel IBV strain. It should be worth mentioning that new vaccine development approaches are obviously required in order to respond to disease outbreaks in a safe and timely manner.

# Conclusion

Infectious bronchitis is a serious poultry disease with economic implications that mostly impacts countries with large poultry production (Bande *et al.* 2017), but it also affects poultry sectors globally (Jackwood and Wit 2013).

Currently, IBV strains are categorized using a phylogenybased categorization approach developed by Valastro and colleagues. The phylogenetic tree given in Fig. 1 shows the antigenic diversity of S1 gene of infectious bronchitis virus in South Asian region. The IBV variants or strains are currently classified into eight genotypes (GI-GVIII), 39 different lineages (GI-1 to GI-31, GII-1, GII-2, GIII-1, GIV-1, GV-1, GVI-1, GVII-1 and GVIII-1) as well as a large number of inter-lineage recombinants that are not yet classified. Most IBV lineages are limited to specific geographic regions, while certain countries report the circulation of distinct lineages. On the other hand, the lineages GI-1, GI-13, GI-16, and GI-19 are widely distributed (Krisztina et al. 2022). The study conducted in Pakistan, evaluated the criteria using the 9 novel sequences for all Pakistani isolates of IBV. Eigth sequences of IBV isolates out of nine were grouped in GI-13 and one was placed in GI-24 lineage (Saleem et al. 2024). It suggested that Different IBV strains isolated and identified in Pakistan are diverse in terms of genetics, offering a base for recombination and challenging the biological control protocols. Another study showed that IBV strain (Pak-973) has diverse mutations when matched with Mass and 4/91 strains which are being used as vaccine strains. The sequenced isolate Pak-973 varied from other serotypes by an average of 7%. These observable changes or substitutions in HVR1 and HVR2 ensure that this isolate is distinct from the vaccination strains that are used in vaccines. This demonstrates the importance of vaccine matching strategy before choosing a strain to use as a vaccine seed when introducing a novel IBV vaccination strain in a particular area. Conclusively, the major factors involved in the prevalence of Infectious bronchitis are occurrence of multiple strains of IBV, lower level of humoral immunity and emergence of novel IBV strains. This review presented a little contribution in demonstrating the similarity index between Pakistani IBV and strains of selected south Asian countries. This similarity index was established through phylogenetic analysis of different IBV strains. Furthermore, A detailed summary of the most recent findings regarding the genetic group distribution of avian coronavirus which may be helpful in vaccine production for different IBV strains circulating in selected South Asian countries. It is very important that vaccine matching should be done before vaccination. Vaccine matching is crucial for maintaining the efficacy of vaccines, as it allows researchers and healthcare professionals to stay ahead of the evolving nature of infectious agents and address emerging strains that might pose challenges to existing vaccine formulations.

# Acknowledgement

I would like to express my sincere gratitude to the senior colleagues at the National Institute for Genomics and Advanced Biotechnology, NARC, Islamabad, for their invaluable guidance and support throughout the process of writing and analyzing the review paper. Their expertise and insights have significantly contributed to the quality and depth of this manuscript. I am also thankful to all my coauthors for their collaboration and input, which have enriched the content of the review paper. This work would not have been possible without their collective effort and encouragement.

### **Author Contributions**

BJ: Complete write up of original Article and Phylogenetic Analysis and collection of relevant data, TK: Idea and conceptualization, FA: Visualization (presentation of data, such as creating figures or tables), MNR: technical Support, HMBA: Proof-reading of review paper.

#### **Conflicts of Interest**

The authors have no conflicts of interest to declar.

#### **Data Availability**

The data used in this study can be obtained from the corresponding author upon a reasonable request.

#### **Ethics Approval**

Not applicable

#### References

- Abro SH, LH Renström, K Ullman, S Belák, C Baule (2012a). Characterization and analysis of the full-length genome of a strain of the European QX-like genotype of infectious bronchitis virus. Arch Virol 157:1211–1215
- Abro SH, K Ullman, S Belák, C Baule (2012b). Bioinformatics and evolutionary insight on the spike glycoprotein gene of QX-like and Massachusetts strains of infectious bronchitis virus. *Virol J* 9:211
- Ahmed Z, K Naeem, A Hameed (2007). Detection and seroprevalence of infectious bronchitis virus strains in commercial poultry in Pakistan. *Poult Sci* 86:1329–1335
- Anonymous (2004). OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Mammals, Birds and Bees), 5<sup>th</sup> edn. World Organization for Animal Health, Paris, France
- Bande F, SS Arshad, AR Omar, M Hair-Bejo, A Mahmuda, V Nair (2017). Global distributions and strain diversity of avian infectious bronchitis virus: A review. *Anim Health Res Rev* 18:70–83
- Bayry J, MS Goudar, PK Nighot, SG Kshirsagar, BS Ladman, JG Jr, GN Kolte (2005). Emergence of a nephropathogenic avian infectious bronchitis virus with a novel genotype in India. J Clin Microbiol 43:916–918
- Bhuiyan ZA, MZ Ali, MM Moula, M Giasuddin, ZUM Khan (2019). Prevalence and molecular characterization of infectious bronchitis virus isolated from chicken in Bangladesh. *Vet World* 12:909–915
- Bickerton E, HJ Maier, P Stevenson-Leggett, M Armesto, P Britton (2018). The S2 subunit of infectious bronchitis virus Beaudette is a determinant of cellular tropism. J Virol 92:10–27
- Cavanagh D (2007). Coronavirus avian infectious bronchitis virus. Vet Res 38:281–297
- Cavanagh D, S Naqi (2003). Infectious bronchitis. Dis Poult 11:101-119
- Chen L, B Xiang, Y Hong, Q Li, H Du, Q Lin, C Xu (2021). Phylogenetic analysis of infectious bronchitis virus circulating in southern China in 2016–2017 and evaluation of an attenuated strain as a vaccine candidate. *Arch Virol* 166:73–81

- Chen Y, L Jiang, W Zhao, L Liu, Y Zhao, Y Shao, S Liu (2017). Identification and molecular characterization of a novel serotype infectious bronchitis virus (GI-28) in China. Vet Microbiol 198:108–115
- Elankumaran S, C Balachandran, N Chandran, P Roy, A Albert, R Manickam (1999). Serological evidence for a 793/B related avian infectious bronchitis virus in India. *Vet Rec* 144:299–300
- Ennaji Y, K Khataby, MM Ennaji (2020). Infectious bronchitis virus in poultry: Molecular epidemiology and factors leading to the emergence and reemergence of novel strains of infectious bronchitis virus. *Emerg Reemerg Viral Pathogens* 2020:31–44
- Fan WS, HM Li, YN He, N Tang, LH Zhang, HY Wang, T Huang (2018). Immune protection conferred by three commonly used commercial live attenuated vaccines against the prevalent local strains of avian infectious bronchitis virus in southern China. J Vet Med Sci 80:1438–1444
- Farsang A, C Ros, LH Renström, C Baule, T Soos, S Belak (2002). Molecular epizootiology of infectious bronchitis virus in Sweden indicating the involvement of a vaccine strain. Avian Pathol 31:229–236
- Ghalyanchilangeroudi A, H Najafi, MF Mehrabadi, ZZ Kafi, N Sadri, AH Rajeoni, H Hosseini (2020). The emergence of Q1 genotype of avian infectious bronchitis virus in Iran, 2019: The first report. *Iran J Vet Res* 21:230–233
- Ghorbiani M, Z Boroomand, M Mayahi, MRSA Shapouri (2020). Molecular identification of infectious bronchitis virus isolated from respiratory diseases in some Iranian broiler flocks. *Mol Biol Rep* 47:7161–7168
- Habibi M, V Karimi, A Langeroudi, S Ghafouri, M Hashemzadeh, R Farahani, P Seifouri (2017). Combination of H120 and 1/96 avian infectious bronchitis virus vaccine strains protect chickens against challenge with IS/1494/06 (variant 2)-like infectious bronchitis virus. Acta Virol 61:150–160
- Han Z, M Gao, Y Chen, W Zhao, J Sun, Y Zhao, S Liu (2018). Genetics, antigenicity and virulence properties of three infectious bronchitis viruses isolated from a single tracheal sample in a chicken with respiratory problems. *Virus Res* 257:82–93
- Han Z, C Sun, B Yan, X Zhang, Y Wang, C Li, Q Liu (2011). A 15-year analysis of molecular epidemiology of avian infectious bronchitis coronavirus in China. *Infect Genet Evol* 11:190–200
- Jackwood MW (2012). Review of infectious bronchitis virus around the world. Avian Dis 56:634–641
- Jackwood MW, SD Wit (2013). Infectious bronchitis. In: Diseases of Poultry, 13<sup>th</sup> edn, pp:139–159. Swayne DE (Ed.). John Willey & Sons Inc., New York, USA
- Jiang L, W Zhao, Z Han, Y Chen, Y Zhao, J Sun, S Liu (2017). Genome characterization, antigenicity and pathogenicity of a novel infectious bronchitis virus type isolated from south China. *Infect Genet Evol* 54:437–446
- Jordan B (2017). Vaccination against infectious bronchitis virus: A continuous challenge. Vet Microbiol 206:137–143
- Krisztina B, B Adam, B Krisztian (2022). Geographic distribution of IBV lineages. *Hung Vet J* 144: 673–690
- Kwon HM, MW Jackwood (1995). Molecular cloning and sequence comparison of the S1 glycoprotein of the Gray and JMK strains of avian infectious bronchitis virus. *Virus Genes* 9:219–229
- Li L, C Xue, F Chen, J Qin, Q Xie, Y Bi, Y Cao (2010). Isolation and genetic analysis revealed no predominant new strains of avian infectious bronchitis virus circulating in South China during 2004– 2008. Vet Microbiol 143:145–154
- Liu S, Q Zhang, J Chen, Z Han, X Liu, L Feng, G Tong (2006). Genetic diversity of avian infectious bronchitis coronavirus strains isolated in China between 1995 and 2004. Arch Virol 151:1133–1148
- Ma T, L Xu, M Ren, J Shen, Z Han, J Sun, S Liu (2019). Novel genotype of infectious bronchitis virus isolated in China. Vet Microbiol 230:178–186
- Najafi H, AG Langeroudi, M Hashemzadeh, V Karimi, O Madadgar, SA Ghafouri, RK Farahani (2016). Molecular characterization of infectious bronchitis viruses isolated from broiler chicken farms in Iran, 2014–2015. Arch Virol 161:53–62
- Parvin R, JA Begum, M Nooruzzaman, CK Kabiraj, EH Chowdhury (2021). Circulation of three genotypes and identification of unique mutations in neutralizing epitopes of infectious bronchitis virus in chickens in Bangladesh. Arch Virol 166:3093–3103

- Patel BH, MP Bhimani, BB Bhanderi, MK Jhala (2015). Isolation and molecular characterization of nephropathic infectious bronchitis virus isolates of Gujarat state, India. *Virusdisease* 26:42–47
- Promkuntod N, S Thongmee, S Yoidam (2015). Analysis of the S1 gene of the avian infectious bronchitis virus (IBV) reveals changes in the IBV genetic groups circulating in southern Thailand. *Res Vet Sci* 100:299–302
- Rafique S, K Naeem, N Siddique, MA Abbas, AA Shah, A Ali, A Rahim, F Rashid (2018a). Determination of genetic variability in Avian Infectious Bronchitis Virus (AIBV) isolated from Pakistan. *Pak J Zool* 50:695–701
- Rafique S, N Siddique, MA Abbas, AA Shah, A Sharif, K Naeem (2018b). Isolation and molecular characterization of infectious bronchitis virus (IBV) variants circulating in commercial poultry in Pakistan. *Pak Vet* J 38:365–370
- Raja A, GD Raj, K Kumanan (2020). Emergence of variant avian infectious bronchitis virus in India. *Iran J Vet Res* 21:33–39
- Ren M, J Sheng, T Ma, L Xu, Z Han, H Li, Y Zhao, J Sun, S Liu (2019). Molecular and biological characteristics of the infectious bronchitis virus TC07-2/GVI-1 lineage isolated in China. *Infect Genet Evol* 75:2–12
- Sadri N, A Ghalyanchilangeroudi, MF Mehrabadi, H Hosseini, A Shayeganmehr, M Sediqian, F Mousavi (2019). Genotyping of avian infectious bronchitis virus in Afghanistan (2016–2017): The first report. *Iran J Vet Res* 20:60–63
- Saleem W, N Vereecke, MG Zaman, F Afzal, I Reman, SUH Khan, H Nauwynck (2024). Genotyping and phylogeography of infectious bronchitis virus isolates from Pakistan show unique linkage to GI-24 lineage. *Poult Sci* 103:103236
- Sumi V, SD Singh, K Dhama, V Gowthaman, R Barathidasan, K Sukumar (2012). Isolation and molecular characterization of infectious bronchitis virus from recent outbreaks in broiler flocks reveals emergence of novel strain in India. *Trop Anim Health Prod* 44:1791–1795
- Sun L, X Tang, J Qi, C Zhang, J Zhao, G Zhang, Y Zhao (2021). Two newly isolated GVI lineage infectious bronchitis viruses in China show unique molecular and pathogenicity characteristics. *Infect Genet Evol* 94:105006
- Uddin M, M Islam, T Rakib, S Das, K Kamaruddin, P Biswas (2016). Molecular detection of infectious bronchitis virus isolated from commercial breeder farms in Chittagong District, Bangladesh. *Adv Anim Vet Sci* 4:370–375
- Valastro V, EC Holmes, P Britton, A Fusaro, MW Jackwood, G Cattoli, I Monne (2016). S1 gene-based phylogeny of infectious bronchitis virus: An attempt to harmonize virus classification. *Infect Genet Evol* 39:349–364
- Wit JD, A Malo, JK Cook (2019). Induction of IBV strain-specific neutralizing antibodies and broad spectrum protection in layer pullets primed with IBV Massachusetts (Mass) and 793B vaccines prior to injection of inactivated vaccine containing Mass antigen. Avian Pathol 48:135–147
- Wit JD, JK Cook, HMVD Heijden (2011). Infectious bronchitis virus variants: A review of the history, current situation and control measures. Avian Pathol 40:223–235
- Worthington KJ, R Currie, RC Jones (2008). A reverse transcriptasepolymerase chain reaction survey of infectious bronchitis virus genotypes in Western Europe from 2002 to 2006. Avian Pathol 37:247–257
- Xu G, J Cheng, S Ma, W Jia, S Yan, G Zhang (2018). Pathogenicity differences between a newly emerged TW-like strain and a prevalent QX-like strain of infectious bronchitis virus. *Vet Microbiol* 227:20–28
- Zhang X, T Deng, J Lu, P Zhao, L Chen, M Qian, Y Wang (2020). Molecular characterization of variant infectious bronchitis virus in China, 2019: Implications for control programmes. *Transb Emerg Dis* 67:1349–1355
- Zhao Y, H Zhang, J Zhao, Q Zhong, JH Jin, GZ Zhang (2016). Evolution of infectious bronchitis virus in China over the past two decades. J Gen Virol 97:1566–1574
- Zulperi ZM, A Omar, S Arshad (2009). Sequence and phylogenetic analysis of S1, S2, M, and N genes of infectious bronchitis virus isolates from Malaysia. *Virus Gene* 38:383–391