Review Article

Molecular Biology, Immunosuppression and Pathogenesis of Infectious Bursal Disease Virus

Altaf Hussain[1]*, Muhammad Saad Ashraf[3], Asim Shamim[1], Mohsin Nawaz[1], Arslan Mehboob[2] and Urooj Fatima[2]

[1]Faculty of Veterinary and Animal Sciences, University of Poonch Rawalakot Azad Jamu and Kashmir, Pakistan; [2]University of Veterinary and Animal Science Lahore, Pakistan; [3]THQ Civil Hospital, Daska, Sialkot, Pakistan.

Abstract | Infectious bursal disease (IBD), caused by infectious bursal disease virus (IBDV), is one of the most devastating and immunosuppressive diseases of the poultry and has been a constraint on the sustainable food security around the globe including Pakistan. Poultry industry is the second biggest industry in Pakistan while IBD is an important disease seriously threatening poultry farming. Despite the use of mass and intense vaccination regimens, the disease continues to sustain in many countries around the globe including Pakistan. However, to some extent, the epidemic strains and epidemic patterns predominant strains of IBDV in Pakistan is unclear. This highlights the need to characterize field strains of IBDV to ascertain the evidence of vaccine failure and to establish foundations for the development of vaccine matching with the epidemic strains.

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***Correspondence** | Altaf Hussain, Faculty of Veterinary and Animal Sciences, University of Poonch Rawalakot Azad Jamu and Kashmir, Pakistan; **Email:** draltafvirk@upr.edu.pk
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Introduction

The causative agent of infectious bursal disease (IBD) is infectious bursal disease virus (IBDV), which is the caustivie agent of acute, highly contagious infection affecting commercial poultry worldwide (Müller *et al*., 2003; Qin and Zheng, 2017). IBD was first identified in 1957 in Gumboro, which is located in the Delaware State of USA and was given the name 'Gumboro'. IBD destroys bursa of Fabricius between 3rd to 6th week of age, hence the disease is known as "AIDS" of poultry (Lasher and Shane, 1994). The clinical picture of the disease includes a sudden onset with a short incubation period, anorexia, weakness, tremors, and intermittent diarrhea (Becht and Müller, 1991; Kibenge *et al*., 1988). Characteristic necropsy lesions include dehydration skeletal muscle hemorrhages. The disease causes severe immunosuppression along with high mortality which leads to a higher incidence of secondary infections (Quaroni and Calnek, 1999). In 1970, the disease was officially named as the infectious bursal disease by World Poultry Congress. IBD was controlled easily before 1980s, as classic strains of IBDV (cIBDV) used in vaccines produced good immune protection and low mortality. However, the situation of controlling IBD was totally changed at the end of the 20th century. Variant strains of IBDV (varIBDV) emerge (Jackwood and Saif, 1987; Rosenberger and Cloud, 1986), which antigenically differ from cIBDV strains resulting in failure of immunization with older strains. After that, extremely virulent strains of IBDV named as vvIBDV were emerged due to the continuous evolution of virus which showed more than 60% mortality rate (Chettle

et al., 1989; Eterradossi *et al*., 1997) resulting in huge economic losses to farmers due to the increased cost of prevention. Now, vvIBDV has a worldwide prevalence, which constantly poses a great threat to the poultry industry. IBD is listed as "important disease of animals which greatly affect the social economy" by World Organisation for Animal Health (OIE).

Epidemiology of IBD

IBDV can infect turkeys and chickens but clinical signs and pathological lesions are only prominent in chickens (Eterradossi and Saif, 2013). IBDV have two serotypes: Serotype-I only infect chickens and is highly pathogenic while serotype-II infects turkeys and non-pathogenic. The disease has a sudden onset and rapid expansion with no effect of season on its endemicity. The disease can be transmitted to other flocks from sick birds through a direct or indirect route. Virus-infected bird sheds a large number of virions in their feces which can be transmitted within the flocks. The virus can withstand acid treatment (at pH=2 for 1 hour), heat resistant (56°C for 5 hours) favouring the pathogeneicity or infectivity *in vivo* and *in vitro*. The virus can directly infect feeding utensils, feed, and water for a longer duration while indirectly virus is transmitted by beetles or rodents which are reservoirs of disease transmission. Due to constant evolution of virus and variations in environmental circumstances, IBD has certain new features:

1. Disease course is prolonged with widened age of onset. Day old chicks, as well as late laying birds, can develop the disease. The disease can persist for more than 2 weeks.
2. Virus-host spectrum is widened. Geese, ducks, and sparrows have found naturally infected with IBDV particularly in ducks seroprevalence rate was 95.5% (Ruiz-Hernandez *et al*., 2016).
3. Natural mutation and recombination of IBDV constantly occurs while currently, vvIBDV is more popular in the poultry sector. Epidemiological studies reported segmental reassortment of IBDV and also indicated that the currently popular infectious bursal disease virus is a new threat (Lu *et al*., 2015).
4. Vaccine immunization failure have occurred. Continuous evolution of virus is a great challenge in the development of new effective vaccines.
5. Mixed IBDV epidemics are reported. Immunity of virus-infected chickens is decreased due to increased virus virulence, concurrent or secondary infections with Marek's virus (MDV), Newcastle disease virus (NDV), Mycoplasma, and bacteria.

Infectious bursal disease virus (IBDV)

Classification and morphological structure: IBDV is placed in genus Avianbirnavirus, family Birnaviridae (Dobos, 1979; Müller *et al*., 2003). Mature virus particles have single layered capsid without envelope. Mature virus particles are icosahedral spheres having T=13 symmetry with 72 nm extended diameter of the fifth-order axis and 66 nm extended diameter of the cubic axis as shown by negatively stained electron microscopy. The viral proteins such as VP1, VP2, VP3, and VP4 shares 3%, 51%, 40%, and 6% of the total quality of IBDV (Dobos, 1995). VP2 have 780 subunits, VP3 have 600 while VP4 have 60 subunits, respectively. There are 260 trimers formed by VP2 monomers which formed the outer capsid of the virus while the inner surface of IBDV has several Y-shaped structures, VP4 might wrap fifth axis edge while VP2 and VP1 both interact with RNA. Non-structural VP5 doesn't participate in the composition of the mature virus.

Genomic structure

Segment A and segment B are two double-stranded segments of IBDV genome. Length of segment A is approximately 3.2 kb and contains two differently sized open reading frames (ORFs). Small open reading frame (ORF) encodes 17kDa non-structural protein named VP5 (Mundt *et al*., 1995) while large ORF encodes 110 kDa precursor polyprotein (NH3-Pvp2-VP4-VP3-COOH) (Azad *et al*., 1985; Hudson *et al*., 1986), which was converted to VP2 (41kDa), VP3 (32 kDa) and VP4 (28 kDa) after post-translational modifications by proteolytic action (Tacken *et al*., 2003). Segment B has single ORF and is about 2.8 kb long which encodes 95 kDa VP1 protein having RNA dependent RNA polymerase activity (Spies *et al*., 1987; von Einem *et al*., 2004).

The 3' and 5' end of both segments A and B are untranslated regions (UTR) while VPg is covalently attached to 5' end of both segments (Müller and Nitschke, 1987). Alignment of different sequences indicated that the presence of 32 bp long highly conserved region at 5' UTR of both segments and studies have proved this to be a promoter sequence (Nagarajan and Kibenge, 1997). In 20 bp upstream of the start codon, there is polypyrimidine region in 5'UTR which is complementary to 3' end region of 18S rRNA (Mundt and Müller, 1995) which may

have a role in the initiation of protein translation. There is a continuous 4 to 5 cytosine ends in 3' UTR of both segments and studies have indicated a possible inhibitory role of this cytosine in replication of the virus (Boot *et al*., 1999). The untranslated region of IBDV can form a secondary stem-loop structure which has a role in UTR functioning (Boot and Pritz- Verschuren, 2004).

Evolution of IBDV

IBD was first identified nearly six decades ago and still, it is considered havoc to commercial poultry (Eterradossi and Saif, 2013). IBDV has two antigenically distinct serotypes: serotype 1 is highly pathogenic to chickens while serotype 2 is non-pathogenic (Ismail *et al*., 1988; Wang *et al*., 2007). Initially, after the first report of IBDV in 1957, virus strains were less virulent and easily controlled. However, virulent strains having the capability to cause disease despite immunization with classical strains were reported in 1985 in USA (Snyder *et al*., 1988). Virus neutralization (VN) test indicated that these strains were different from older classical IBDV strains (Jackwood and Saif, 1987). Very virulent strains of IBDV (vvIBDV) were identified in Europe in 1987 and rapidly spread throughout the world (Berg, 2000). Mortality rate was almost over twice as compared to classical virulent strains (cvIBDV) and cause disease even the presence of high titers of maternal antibodies (Eterradossi *et al*., 1992; Van den Berg *et al*., 1991). Based on antigenic, pathotypic, and genetic characteristics, IBDV strains of serotype 1 are categorized into four groups named as classical virulent strains, antigenic variant strains, very virulent strains, attenuated strains.

Coding proteins

VP1

VP1 is the sole protein coded by segment B with approximately 878 long amino acid sequence. VP1 has multifunctions and exist in two forms in virions *i.e.* VPg covalently linked to viral genome and free VP1 (Calvert *et al*., 1991; Müller and Nitschke, 1987). Free VP1 protein is involved in replication and transcription of IBDV through its RNA-dependent RNA polymerase activity (von Einem *et al*., 2004). Covalent bonding of VP1 to the viral genome is mainly due to its self-guanylation activity which helps to bind itself with 1 or 2 guanine (G) bases. Resultant VP1-GG or VP1-G is complementary to cytosine present at 3'-end of viral RNA and acts as protein primer (Dobos, 1995), therefore, is involved in initiating new RNA strand synthesis which has the covalent binding of VP1 (Magyar *et al*., 1998; Xu *et al*., 2004). Studies have confirmed that self-guanylation is not dependent on VP1 polymerase activity nor on RNA template (Pan *et al*., 2009). On the basis of structural morphology, there are three functional domains of VP1 protein: N-terminal domain (aa 1 to 167), central polymerase active region (aa 168 to 658), and C-terminal domain (aa 659 to 878) (Pan *et al*., 2007). Central polymerase active region is right-handed shaped consisting of finger, thumb, and palm which have structural resemblance with active regions of other RNA polymerases. Two reasons make VP1 an important subgroup of RNA polymerases: Firstly, the palm of VP1 contains 5 catalytic motifs (their order CABDE is different from an order of classical strains i.e., ABCDE); secondly, there is a 401ADN403 sequence in C-motif of VP1 protein of IBDV instead of classical X(G)DD sequence. Only two aspartic acid residues (D402 and D416) are present in IBDV RNA polymerase active site (Gorbalenya *et al*., 2002). It was verified that the amino acid triplet at positions 4 and 145/146/147 of VP1 are important determinant of viral replication and pathogenicity (Gao *et al*., 2014; Yu *et al*., 2013). The substitution of these three amino acids could also affect the polymerase activity of IBDV RdRp (Qi *et al*., 2016).

VP2

The nucleotides 131 to 1453 in segment A encoded 441 amino acids of VP2 protein which is the main structural unit of viral capsid identified by neutralizing antibodies (Qi *et al*., 2015a, 2016). X-ray crystallographic studies have shown that VP2 protein folding forms three distinct domains base domain (B), shell domain (S), and protuberance domains (Projection domain, P), in which the B domain and the S domain are composed of the conserved N-terminal and C-terminal amino acids of VP2, and the prominent P domain consists of the amino acid of the VP2 hypervariable region (HVR, aa 206-350), which is located at the outermost side of the P domain. Two protruding rings P_{BC} and P_{HI} are composed of a first hydrophilic region (aa 212-224) and a second hydrophilic region (aa 314-324), respectively (Coulibaly *et al*., 2005; Garriga *et al*., 2006; Letzel *et al*., 2007). The terminus, wherein the amino acid of the first hydrophilic region, is primarily involved in stabilizing the spatial conformation of the

epitope, and the amino acid mutation in the second hydrophilic region is critical for immune escape of IBDV (Heine *et al*., 1991).

In addition, VP2 is also an important virulence protein of IBDV. There are two four loop structures, P_{BC}, P_{HI}, P_{DE} and P_{FG}, at the top of the P domain of VP2. Amino acids 253 and 284 are located at P_{DE} and P_{FG}, respectively. These two amino acid sites can determine cell-tropism of IBDV. It is well known that chicken bursa B lymphocytes are the main target cells of IBDV, and have obvious susceptibility to IBDV. Lymphocyte susceptibility in other organs (such as blood and spleen) is relatively low. This cellular tropism is not limited to the preference of the same strain for B lymphocytes of different tissues, but also for the tropism of virulent and attenuated strains to different types of cells. Studies have shown that IBDV wild-type strains or virulent strains can rapidly proliferate on bursal B lymphocytes in vivo, but cannot replicate on *in vitro* cell such as CEF and DF1; unlike IBDV wild strains, IBDV attenuated strains can not only adapt to bursal B lymphocytes but also adapt to CEF, DF1, Vero, and other cells for *in vitro* propagation. With the development of reverse genetic technology, the molecular determinant of the cell-tropism difference of virulent and attenuated IBDV has gradually been revealed.

At the beginning, VP2 has shown to be the single most important determinant of cell-tropism of IBDV (Boot *et al*., 2000; Brandt *et al*., 2001) and its amino acid residues 253, 279, 284 and 330 have been studied. While residue 330 has little influence on the ability of IBDV to infect tissue culture, the roles of the residues 253, 279, 284 in cell-tropism are conflictive (Brandt *et al*., 2001; Lim *et al*., 1999; Mundt, 1999; Van Loon *et al*., 2002). In one blind-paasage experiment in Harbin Veterinary Research Institute, Prof. Wang Xiaomei's team found that only two amino acid mutations difference (Q253H and A284T) in VP2 was found between vvIBDV Gx strain (not adapt to CEF cell) and one intermediate strain CEF-9 that was partially attenuated and adapted to the CEF culture (Wang *et al*., 2004). Subsequently, Qi *et al*. systematically verified the molecular basis of IBDV cell tropism by using the novel reverse genetics technology and finally confirmed the double mutation of of Q253H/A284T on VP2 can adapt to vvIBDV to cell lines while single mutation cannot (Qi *et al*., 2009). Meanwhile, it was also verified that the double mutation of of Q253H/A284T can attenuated vvIBDV (Qi *et al*., 2009). Furthermore, the study also confirmed that 222, 249, 256, and 279 amino acids on VP2 have been involved in the replication and virulence of IBDV (Brandt *et al*., 2001; Li *et al*., 2016; Lim *et al*., 1999; Qi *et al*., 2013, 2014, 2015a).

VP3

The nucleotides 2393-3169 form segment A encoded 257 amino acids of VP3 protein which is located on the inner surface of virus particles. VP3 protein itself can't initiate production of virus-specific neutralizing antibodies. Morphological studies of the VP3 gene showed that it is mainly composed of α-helices connected by rings of different sizes. VP3 protein has two distinct domains: N-terminus (first domain) and hydrophobic C-terminus (second domain) which has resemblance with its oligomerization. VP3 can exist in the form of dimmer and has high structural resemblance with the structure of transcriptional regulatory factor proteins which indicates that it has some roles in the regulation of transcription (Maraver *et al*., 2003). C-terminus is responsible for several ancillary functions performed by VP3 protein due to which it is also called "moonlight protein". C-terminus of VP3 interacts with VP1 protein (Casañas *et al*., 2008) and can alone activate VP1 protein RNA polymerase activity. This interaction with VP1 protein results in modulation of its spatial conformation which helps viral RNA to enter VP1 protein catalytic center (Ferrero *et al*., 2015; Garriga *et al*., 2007). This interaction also results in the production of mature virus particles. Studies have shown that in the absence of C-terminus of VP3, IBDV has a rod-like structure instead of globular icosahedral structure. This effect is attributed to the presence of the last glutamate (Glu257) at VP3 C-terminus (Chevalier *et al*., 2004; Maraver *et al*., 2003). C-terminus of VP3 have a role in antigenicity and virulence of IBDV. Studies have shown that C-terminus of IBDV serotype-I replaced by serotype-II results in different virus antigenicity and virulence (Boot *et al*., 2002). Virulence of IBDV is decreased if C-terminus at end of VP3 proteins is replaced by attenuated strain containing Val235 (Wang *et al*., 2010). The C-terminus of VP3 can also has the ability to bind with single-stranded RNA (Maraver *et al*., 2003) while 99Lys-102Arg-105Lys-106 Lys of VP3 protein can bind to double-stranded RNA. This interaction with double-stranded RNA may inhibit the production of interferons (Ye *et al*., 2014) It was peported that a single amino acid

at 990 in the C-terminus of VP3 protein influences the replication of attenuated infectious bursal disease virus *in vitro* and *in vivo* (Wang *et al*., 2010).

VP4

VP4 protein consists of 242 amino acids encoded by nucleotides 1667-2392 of segment A, is present in small amounts in virions, is a non-structural protein, has eukaryotic serine protease hydrolyzing activity, and is capable of self-cleaving precursor polyprotein (NH2-Pvp2-VP4-VP3-COOH) plays an important role in the modification and processing of viral proteins. Studies have shown that the enzyme active sites of VP4 protein are Ser140 and Lys180 (corresponding to Ser652 and Lys692 on polyprotein, respectively), and the cleavage site corresponds to 511Leu-512Ala^513Ala and 754Met-755Ala^756Ala on the polyprotein (Lejal *et al*., 2000). Recent studies have found that VP4 protein is also a phosphorylated protein, Ser26, Tyr99, Thr162 (corresponding to amino acids 538, 611, and 674 on the polyprotein, respectively) are the major phosphorylation sites. It plays a role in the protease activity of VP4 (Wang *et al*., 2015b). In addition, VP4 also has an impact on the self-assembly of IBDV (Lee *et al*., 2015).

VP5

VP5 protein consists of 145 amino acids encoded by the small segment A ORF (nucleotides 97-534). It is a non-structural protein and is not found in virions (Mundt *et al*., 1995). It has been reported that VP5 is a replication-non-essential protein (Mundt *et al*., 1997). However, based on reverse genetics, it was verified that VP5 played an important role in viral replication and pathogenesis both *in vitro* and *in vivo* (Qin *et al*., 2009, 2010). Although VP5 protein has little effect on viral replication, there is increasing evidence that VP5 protein is involved in the release of progeny virions. Lombardo *et al*. (2000) found that VP5 protein can accumulate around the cell membrane and induce cell lysis (Lombardo *et al*., 2000; Wu *et al*., 2009). The literature reported that VP5 is a phosphoinositide-binding protein and demonstrated that VP5 protein is essential for the spread of the virus among cells (Méndez *et al*., 2015). Although topological prediction analysis revealed that VP5 protein may be a type II transmembrane protein, including potential transmembrane and intracellular N-terminal and C-terminal structures, the latest findings clearly indicate that VP5 is not a transmembrane protein (Carballeda *et al*., 2015). Therefore, the role of VP5 protein in the release of virions remains to be further studied. In addition, another important role of VP5 protein is to participate in apoptosis during IBDV infection (Li *et al*., 2012; Lin *et al*., 2015).

Reassortment of IBDV

It has been reported that co-evolution of genome segments is a major evolutionary feature in IBD (Le Nouen *et al*., 2006). However, recently, it has been reported a few strains exhibited markedly different genetic relatives for segments A and B.

vv-A/Att-B

The reassortment category of vv-A/Att-B means that segment A belongs to very virulent IBDV (vvIBDV) while segment B belongs to attenuated strain (attIBDV). SH95 is a vvIBDV isolated in Shanghai from flocks having a history of vaccination failure. A single step PCR successfully amplifies 2827 base pairs (bp) long B segment and 3259 bp long segment A. Amplified product was cloned and then sent for genetic sequencing. 27 amino acid positions in the genome of segment A were substituted while VP1 protein has 9 to 38 substitutions. Four out of 38 substitutions in VP1 while three out of 27 amino acid substitutions in segment A were unique. Alignment and subsequent comparison of sequences revealed the fact that they were most similar to routinely isolated Asiatic vvIBDVs clearly different from classical IBDV strains. The topology tree indicated the possible emergence of SH95 strain through a genetic reassortment with very virulent A and attenuated B (Sun *et al*., 2003b).

In 2011, a novel vvIBDV strain named as GX-NN-L was isolated in the province of Guangxi from broiler birds. This strain caused severe immunosuppression, atrophy of bursa and high mortality in broiler flocks (Chen *et al*., 2012a). In a study, a full genome of natural reassortant strain named GX-NN-L was characterized by having segment A resembled with vvIBDV while segment B related to attenuated IBDV. Findings of this study helped to get important information about the exchange of genetic material between a very virulent strain of IBDV and attenuated IBDV strain which leads to monitor disease spread in commercial chickens (Chen *et al*., 2012a). In another study, virus strain was isolated from the outbreak of IBDV in 2004 in Lusaka (named as KZC-104) and the full genome sequence was determined. The coding region of segment A consisted of 3,074 nucleotides

while the coding region of segment B was 2,651 nucleotides long. Deduced amino acid sequences were aligned and phylogenetic analysis was performed which showed that segment A of KZC-104 genome was derived from vvIBDV strain while segment B resembled with attenuated IBDV strain. Results of nucleotide blast showed 98% nucleotide sequence similarity with D6948 strain which was vvIBDV while 99.8% similarity with D78 (attenuated IBDV strain) (Kasanga *et al*., 2013). In 2015, a new natural reassortant strain of IBDV named as IBD13HeB01 with vv-A/ Att-B was isolated from northern China. In 2017, coding regions of both segments of novel reassortant strain named as JBN2011 were sequenced. JBN2011 genome characterization indicated a rare recombinant virus whose segment A had vvIBDV portion and segment B was related to Bursine-2-like attenuated IBDV (Lee *et al*., 2017).

In a study, an Indian strain named MB11/ABT/MVC/2016 was isolated from commercial broilers and full genome sequence was identified which was first complete genome sequence of IBDV from India (Senthilkumar *et al*., 2016).

Att-A/vv-B

Wei and co-workers characterized the full genome of a reassortant strain of IBDV named ZJ2000 isolated from virus outbreaks in commercial poultry flocks. Alignment of sequences showed ZJ2000 belongs to the category of natural reassortant whose segment A and B derived from attenuated (att) and vvIBDV, rwspectively (Wei *et al*., 2006). ZJ2000 showed delayed replication strategy when compared with attenuated IBDV strains. This strain proved to be pathogenic for SPF chickens when administered experimentally. Challenge of this strain results in 100% morbidity and 26.7% mortality along with significant pathological lesions in bursa of Fabricius. Results also indicated that VP2 alone can't determine the virulence of IBDV and RNA-dependent RNA VP1 protein also have a role in virulence determination (Wei *et al*., 2006). In another study, the complete genome sequence of an IBDV reassortant named TL2004 was characterized and it was revealed that segment A resembles with attenuated IBDV strains while segment B resembles with vvIBDV strains. This strain was also pathogenic to SPF chickens and embryonated eggs but not such virulent as vvIBDV (Wei *et al*., 2008). Besides, phylogenetic analysis showed HN strain isolated in centrol China is also one Att-A/vv-B type, of which segment A was similar with vvIBDV OKYM strain and segment B was similar with attenuated B87 strain (Cui *et al*., 2013).

vv-A/c-B

In this category (vv-A/c-B), segment A is derived from vvIBDV strains and segment B is derived from classical IBDV (cIBDV). In 2003, the first reassortment IBDV (Br/03/DR) was reported, of which segment A is derived from vvIBDV and segment B from cIBDV (Fernandes *et al*., 2012). Another vv-A / c-B strain (CA-S7610) was islotated in America (Gallardo *et al*., 2014).

vv-A/var-B

It is possible that the reassortant might occur between vvIBDV and variant IBDV (varIBDV), which was verified by one special reassortant IBDV (02015.2) isolated in Venezuela. The 02015.2 strain has vv-A / var-B. This strain induced significantly less mortality than typical vvIBDVs (Le Nouen *et al*., 2006).

vv-A/II-B

Natural reassortants were already reported between vvIBDV and various other strains of serotype-I, but between serotype I and II, no natural reassortant has been reported. The vv-A/II-B means segment A belongs to vvIBDV while segment B belongs to serotype II strain. The first case of vv-A / II-B reassortants (CA-D495 and CA-K785 strains) was reported in California in 2009. Segment A of these reassortants resembled with serotype I of vvIBDV but segment B was related to serotype-II. CA-K785 reassortant resulted in 20% mortality but caused no clinical signs in turkeys (Jackwood *et al*., 2011). Besides, another study described the first event of inter-serotypic reassortment in Europe. The isolated strain was named as 100056 and has segment A related to European vvIBDV strains and segment B related to serotype-II of European isolates. In animal experiment of SPF chickens, 00056 strain iduced notable bursal lesions and atrophy without any significant mortality which suggested the immunosuppressive potential of the isolate (Soubies *et al*., 2017).

vv-A/Uniq-B

In 1996, Harbin Veterinary Research Institute isolated a strain of vvIBDV from Guangxi province in China and named it Gx strain. Subsequently, under the support of the EU cooperation project (ERBIC18CT980330), the Gx strain was identified as

a reference strain of Chinese vvIBDV by international experts of International Epizootic Office (OIE) reference laboratory (Wang *et al*., 2003). Further genome sequencing and genetic evolution analysis revealed that the genomic segment A of Gx strain has higher homology with vvIBDV, but its segment B belongs to a unique branch that is different from vvIBDV and attenuated strain. Another early isolates, Harbin-1, also belongs to this type, and they may be derived from similar ancestors (Hon *et al*., 2008; Xia *et al*., 2008). Recently, another such a type strain HLJ-0504 was isolated and researched in detail (Yu *et al*., 2010). At present, the evolutionary source of this B segment is unknown, so the HLJ0504-like genotype is called vv-A/ uniq B type. The prevalence of HLJ0504-like strains in China is becoming more and more popular. Recently, similar strains have been continuously isolated in Northeast China (Yu *et al*., 2010) and many provinces in the south (He *et al*., 2014). The genome of these strains belongs to vv-A/ uniq B type, but a few mutations have occurred. For example, the HLJ-0504 strain, which shares 99.3% and 92.3% homology with the polyprotein (segment A coding) encoded by the Gx strain and the VP1 protein (segment B coding) (Qi *et al*., 2011). In addition, although the VP5 protein of HLJ-0504 has high homology with vvIBDV, there is a deletion of MLSL peptide at the N-terminus, which is a characteristic of attenuated strains (Qi *et al*., 2011). The vv-A/uniq-B type of IBDV has also been isolated from other countries, such as Venezuela (Le Nouen *et al*., 2006), Nigeria (Nwagbo *et al*., 2016) and Algeria (Abed *et al*., 2018).

Conclusions and Recommendations

Poultry is the second largest industry of Pakistan while IBDV is an important disease threatening poultry farming in the country. While extensive vaccination programs are implemented in the country, the disease outbreaks are not uncommon. This highlights the need to characterize field strains of IBDV to ascertain the bases of vaccine failure and to establish foundations for indigenous virus utilization in the vaccine development. However, limited information about the spread of disease is available in Pakistan which can discover true status of IBDV.

Novelty Statement

It is of paramount significance to characterize field strains of IBDV in order to determine the causes of vaccine failures and establish foundations for indigenous virus utilization in the vaccine development. With the scarcity of available literature in this regard, it is concluded that vaccine alone cannot provide complete protection, rather there is a need to adopt a holistic approach for protection involving vaccine antigen matching, biosecurity, and feeding management.

Author's Contribution

All authors contributed equally in all steps of the research *i.e.* from data collection to write up.

Conflict of interest

The authors have declared no conflict of interest.

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