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Six decades of infectious bursal disease in poultry: The journey so far and challenges ahead

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Abstract

Despite six decades of concerted efforts, Infectious bursal disease (IBD) still remains a major threat to the poultry industry worldwide. Most importantly, the emergence of variant and very virulent strains of infectious bursal disease virus (IBDV) has dramatically changed the epidemiology of the disease, thus resulting in the renewed efforts in the search for effective control measures. Currently, live attenuated, inactivated, and immune-complex vaccines are among the immune-therapeutic approaches employed for the control of IBD in the field alongside adequate biosecurity, albeit with various degrees of success and limitations. Progress in genetic engineering has allowed the generation of reverse genetic IBDV mutants, recombinant live viral vectors expressing the IBDV VP2 immunodominant protein, intra-serotypic recombinant IBDV viral-like particle co-expressing the outer capsid protein structures derived from 2 or more serotype 1 strains or the incorporation of either VP2 or VP2-4-3 polyprotein sequences alongside molecular adjuvants that can be used as IBD vaccine candidates to elicit an immune response. However, despite these advances, outbreaks are still reported even in flocks that have up to date vaccination records and somewhat excellent management practices. This paper reviews aspect of genetic characteristics of IBDV and reflects on the progress and future challenges in providing effective IBD vaccine to achieve effective control of both classical and very-virulent IBDV serotypes that constitute a major devastation to poultry production and health.

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Introduction

Infectious bursal disease is a highly contagious disease with significant economic consequences. It is caused by an RNA virus, the infectious bursal disease virus (IBDV), which is classified in the genus Avibirnavirus of the family *Birnaviridae* (Dobos *et al.*, 1979; Delmas *et al.*, 2019). The disease is highly contagious and acute with severe immunodepression that mainly affects young chickens. The first outbreak of the disease was observed in 1957 and officially reported in 1962 from farms located at the outskirt of

Gumboro community in Sussex County, Delaware, USA (Cosgrove, 1962). During the early outbreaks, the disease was characterized by extensive kidney damage and was then referred to as avian nephrosis (Cosgrove, 1962). The huge economic losses attributed to this disease stemmed from two factors; its severe immunodepressive effects on infected chickens through destruction of IgM bearing developing B lymphocytes in the bursa of Fabricius, a factor that abolishes or reduces the chickens' ability to mount effective humoral immune responses, thus making them more susceptible to other infections and increasing the chances of vaccination failure (Tarpey *et al.*, 2007a) and the high mortality of up to 60% or higher observed in infected young chickens at 3 weeks of age or older. The incubation period of the disease is short (3-5 days) and birds that are less than 3 weeks old do not exhibit clinical signs but are immunosuppressed (Saif, 1991). The degree of immunosuppressive effects of IBDV infection is dependent upon the age of birds at infection with birds at 2 weeks of age being more immunosupressed than at later ages (Saif, 1991).

Two serologically distinct serotypes have been recognized, the pathogenic serotype 1 strains that manifest clinical disease in chickens and the apathogenic serotype 2 viruses that infect turkeys (Becht *et al.*, 1988; Kibenge *et al.*, 1988; Yamazaki *et al.*, 2017) without clinical disease. Additionally, different antigenic sub-types exist within the IBDV serotype 1 viruses (Liu *et al.*, 1994; Ching *et al.*, 2007). The early strains called classical (ca) IBDV, induced bursa enlargement due to inflammation followed by atrophy, while the variant strains emerged in the early 1980s and caused only bursal atrophy without inflammation (Saif, 1991) and the very virulent strains that emerged in the late 1980s characterized bursal enlargement followed by atrophy and high mortality (Berg, 2000). Interestingly, immunization with one subtypes evokes little or no neutralizing immunity against other subtypes especially when low vaccine doses are being administered (Jackwood *et al.*, 1987). Although vaccination and adequate biosecurity had been the most effective control measures against IBD (Muller *et al.*, 2012; Gelb *et al.*, 2016), the emergence of variant and very virulent IBDV pathotypes in the 1980s (Berg, 2000) and the recent distinct IBDVs (dIBDV) (Tomás *et al.*, 2015; Hernández *et al.*, 2015) threatens the effectiveness of the current available vaccines (Alfonso-Morales *et al.*, 2015). These variant and very-virulent types have been observed to break through maternally derived antibody (MDA) and cause infection in young chickens with mortality up to 60% in pullets and 25% in broilers (van den Berg *et al.*, 1991). Most of the available vaccines in use for the control of IBD are either killed or live vaccines having various degrees of efficacies. Young chickens gain protection against IBD infection through maternally derived antibody (MDA) acquired from the dam vaccinated with both killed and live attenuated vaccines (Tarpey *et al.*, 2007b; Tarpey & Huggins, 2007). However, the MDA interferes with the effectiveness of live attenuated vaccines except when intermediate and hot vaccines are used which have various degrees of consequences viz a viz immunosupression due to vaccine-induced bursal damage (Tirziu *et al.*, 2010). Thus, there is an urgent need to develop vaccines that are safe and effective and possibly those able to induce the right immune response even in the presence of MDA (Lawal *et al.*, 2017).

Molecular Biology

Aetiology and genomic organization

Infectious bursal disease virus (IBDV) is a member of the genus Avibirnavirus that belongs to the family Birnaviridae together with the Aquabirnavirus, Blosnavirus and *Entomobirnavirus* genera with each genus having from 1 to 3 species respectively (van Cleef *et al.*, 2014; Smith *et al.*, 2015a; Delmas *et al.*, 2019). This family contains segmented, double-stranded RNA (dsRNA) genome with large and small bisegments designated A and B, enclosed in a naked, single-shelled icosahedral capsid 58 nm to 60 nm in diameter (Dobos *et al.*, 1979; Müller *et al.*, 1979). The smaller B segment (2.7 kb to 2.9 kb) encodes viral protein 1 (VP1 of size of 95 kDa), an RNA-dependent RNA polymerase (RdRp) (Macreadie & Azad, 1993; Jackwood & Stoute, 2013). Segment A (2.9 kb to 3.4 kb) has two partially overlapping open reading frames (ORFs) (Mundt *et al.*, 1995; Tacken *et al.*, 2003; Escaffre *et al.*, 2013).

The first, smaller ORF encodes non-structural viral protein VP5 17 KDa in size, a protein not vital for in vitro viral replication but essential for virus-induced pathogenicity (Mundt *et al.*, 1995; Mundt *et al.*, 1997; Letzel *et al.*, 2007; Carballeda *et al.*, 2015) and cell to cell spread of the virus during infection (Mendez *et al.*, 2015). The larger ORF encodes a polyprotein 110-kDa in size, which by autocleavage, results in three polypeptides: pVP2 (48 kDa), VP3 (32 kDa), and VP4 (28 kDa) (Oña *et al.*, 2004; Luque *et al.*, 2007). This self-processing is VP4 mediated (Petit *et al.*, 2000; Wang *et al.*, 2010), a protein with serine-lysine protease activity (Brown & Skinner, 1996; Birghan *et al.*, 2000; Petit *et al.*, 2000). It is a soluble protein mainly associated with type II tubules of 24 nm in diameter (Granzow *et al.*, 1997). Further processing

of pVP2 (residues 1 to 512) at its carboxy-terminus leads to VP2 (40 kDa) (Da Costa *et al.*, 2002; Tacken *et al.*, 2003; Chevalier *et al.*, 2005) and four other smaller peptides (residues 442 to 487, 488 to 494, 495 to 501, and 502 to 512) three of which (residues 442 to 487, 488 to 494, and 502 to 512) are reported to be associated with the viral particles whose absence affect virus growth (Da Costa *et al.*, 2002; Lee *et al.*, 2004). Virus recovery was shown to be inhibited when the domain of amino acid residues 442 to 487 or 502 to 512 was deleted using reverse genetics and IBDV viability appeared to be associated with several amino acids of the smaller peptide 502 to 512 (Da Costa *et al.*, 2002) (Figure 1).

The putative RNA dependent RNA polymerase (VP1)
The VP1 is present in the virion both as free, and a genome-linked protein (VPg) attached to the 5' end of the positive strands of the two genomic segments (Chevalier *et al.*, 2005; Escaffre *et al.*, 2013; Jackwood & Stoute, 2013). Studies indicated that the VP1 gene sequences of the vvIBDV pathotypes formed a distinct cluster (Jackwood & Stoute, 2013), evidence suggesting that it may arise from genetic reassortment of the B segment (Wei *et al.*, 2006). The VP1 protein modulates viral virulence due to its role in viral replication efficiency (Liu & Vakharia, 2004) as demonstrated by replication inhibition through DNA vector-based RNA interference, directed towards the protein in vitro (Gao *et al.*, 2008; Li *et al.*, 2013).

The major structural protein (VP2)
This protein constitutes about 51% of the total viral proteins (He *et al.*, 2009). At least two neutralizing epitopes are located on this polypeptide that induces the production of virus-neutralizing antibodies (Abs) that protect susceptible chickens from infection (Böttcher *et al.*, 1997; Lazarus *et al.*, 2008). It is responsible for antigenic variation, tissue culture adaptation and viral virulence (Lazarus *et al.*, 2008; Jackwood & Stoute, 2013). Two of the short peptides products of VP2 maturation are important in determining the geometry of the viral particle during assembly and disruption of the cellular plasma membrane during virus attachment and intracytoplasmic translocation (Chevalier *et al.*, 2005; Luque *et al.*, 2007). The protein is folded into three key structural domains: the base, shell and projection (Coulibaly *et al.*, 2005; Letzel *et al.*, 2007; Luque *et al.*, 2007; Coulibaly *et al.*, 2010). The base and shell domains are formed by the conserved N- and C-termini of VP2 (Bayliss *et al.*, 1990; Lombardo *et al.*, 2000). The projection domain is formed by the hypervariable region of VP2 starting from amino acids (AA) position 206 to 350 (Bayliss *et al.*, 1990). Within the VP2 region, two hydrophilic regions A and B have been identified (Jackwood & Sommer, 2002; Adamu *et al.*, 2013). Structurally, region A extends from AA positions 212 to 224, and B spans AA 314 to 325 (Upadhyay *et al.*, 2011). These regions constitute two loops, PBC and PHI (neutralising Ab-binding domains), representing the outmost parts of the projection domain (Letzel *et al.*, 2007). Two additional loops were identified in the projection domain, PDE and PFG (Coulibaly *et al.*, 2005). Moreover, the putative AA responsible for virulence and cellular tropism were identified to be glutamine at 253, aspartic acid at 279, and alanine at 284 (Jackwood & Stoute, 2013; Qi *et al.*, 2013). However, VP2 is not the only virulence determinant in vvIBDV as the VP1 protein has been noted as another virulence determinant as well (Qi *et al.*, 2013).

The internal capsid protein (VP3)
VP3 (32 kDa) is the Y-shaped trimers constituting about 40% of the total viral proteins and forming the inner scaffolding of the capsid upon which the viral proteins are assembled (Luque *et al.*, 2007; Luque *et al.,* 2009). It possesses the group-specific and few neutralizing epitopes (Tacken *et al.*, 2000; Kim *et al.*, 2004) and is known to interact with VP1 (Tacken *et al.*, 2000; Tacken *et al.*, 2002) and with the viral genomic material through its carboxy-terminal domain (Tacken *et al.*, 2002; Luque *et al.*, 2009; Wang *et al.*, 2010) during capsid formation. Tacken *et al.* (2002), however, opined that VP3 protein is functionally implicated in the control of viral replication not only through interaction with its carboxyl-terminal but via interaction with virtually all components of the viral particle: itself, VP2, VP1, and the two genomic dsRNAs and that such interaction eventually led to the production of the progeny virus.

The viral autocatalytic protease (VP4)
VP4 (28 kDa) is a viral protease that uses the serine-lysine (Ser-652 and Lys-692) catalytic dyad devoid of ATPase domain to cleave the polyprotein into individual VP2, VP3 and VP4 proteins (Birghan *et al.*, 2000; Lejal *et al.*, 2000; Petit *et al.*, 2000; Castón *et al.*, 2001). It is a soluble protein mainly associated with type II tubules of 24 nm in diameter (Granzow *et al.*, 1997). The sites for the autocleavage between pVP2–VP4 and VP4–VP3 consist of amino acid residues Leu-Ala-Ala at positions 511 to 513 and Met-Ala-Ala at positions 754 to 756 respectively and these locations are responsible for the specificity of the cleavage sites

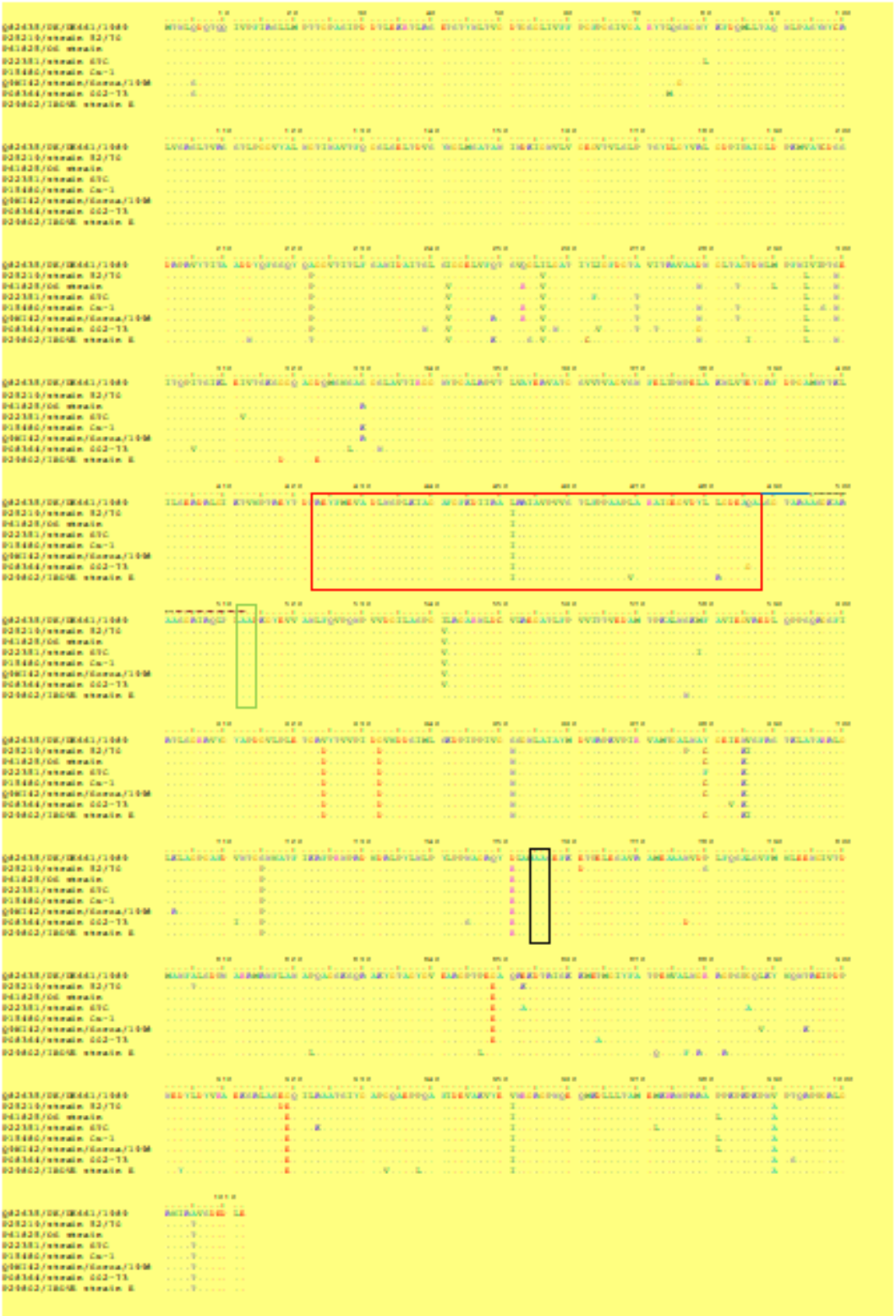

Figure 1: complete amino acid sequences of the IBDV pre-polyprotein gene of isolates representing the vvIBDV (UK661 strain); caIBDV (52/73, OS, STC, Cu-1, Soroa and 002-73 strains) and vaIBDV (Strain E). The polyprotein sequences began with pVP2 (1-512aa), 46 aa residues short peptide (pep1, red box), 11 aa short peptide 2 (pep2, blue line___), 11 aa short peptide 3 (pep3, black round dote….), and 10 aa short peptide 4 (pep4, red dash ----), pVP2-VP4 cleavage site (green box), and VP4-VP3 cleavage site (black box).

and as expected are, therefore, conserved among IBDV serotypes and strains (Sánchez & Rodriguez, 1999). This protein plays a significant role in the pVP2 protein maturation through progressive trimming of several smaller peptides at its C-terminal end during assembly (Lejal *et al.*, 2000).

The viral non-structural protein (VP5)

VP5 is a class II membrane protein with a cytoplasmic N-terminus and an extracellular C-terminal domain (Lombardo *et al.*, 2000; Castón *et al.*, 2001). It is highly basic, cysteine-rich, and semi-conserved among all IBDV serotype 1 strains (Mundt *et al.*, 1995). This protein has been incriminated in the induction of bursal pathology (Yao *et al.*, 1998) and in virus dissemination and release (Méndez *et al.*, 2015; Méndez *et al.*, 2017). The protein accumulates within the cell membrane, resulting in its disruption and decrease in cellular viability. Together with VP2, VP5 has been shown to induce apoptosis in vitro culture (Wei *et al.*, 2011).

Serotypes and pathotypes

Two antigenically distinctive serotypes, 1 and 2, are recognized for IBDV (Kibenge *et al.*, 1988; Lawal *et al.*, 2017; Yamazaki *et al.*, 2017). Viruses in serotype 2 group are naturally neither virulent for chickens nor offer protection against serotype 1 infections (Gallardo *et al.*, 2014), whereas strains of viruses in serotype 1 are pathogenic in chickens with different degrees of virulence, immunosuppression and antigenicity (Boot *et al.*, 2002; Abdul *et al.*, 2013; Stoute *et al.*, 2013). Classical strains of the virus are responsible for the earlier reported outbreaks but in the late eighties, very virulent (vv) strains emerged in Western Europe, Southeast Asia, and Africa that were more virulent than classical strains due to their mortality rates of over 70% (Alfonso-Morales *et al.*, 2013; 2015; Hernández *et al.*, 2015). Similarly, almost at the same time, variant strains emerged in the United States (Zhou *et al.*, 2010), Central America (Hernández *et al.*, 2015) and Australia (Kurukulsuriya *et al.*, 2016) that were antigenically different from the classical or very virulent strains. Classical and very virulent IBDV strains cause hemorrhagic inflammation with severe depletion of bursal follicles but differ only in the mortality rates (30-60% for caIBDV and 70-100% for vvIBDV) (Rasoli *et al.*, 2015). On the other hand, variant IBDV (vaIBDV) strains cause rapid bursal atrophy without evidence of inflammation, haemorrhage and little or no mortality (<10% for vaIBDV) (Kurukulsuriya *et al.*, 2016). They can cause infection in flocks that had been vaccinated with caIBDV-based vaccines (Mahgoub *et al.*, 2012). The emergence of vvIBDV and its global distribution pose an economic threat to the poultry industry worldwide because infection occurs even in flocks with high maternally derived antibody titer against the classical strain even though they share antigenic similarity (He *et al.*, 2016). Isolates of vvIBDV that appeared in Europe, Asia and Africa are genotypically and antigenically similar in the sequences of their VP2 hypervariable region nucleotides and amino acid sequences (Figure 1) and are phylogenetically related (Figure 2) (He *et al.*, 2016).

Genetic Reassortments in IBDV

Antigenic drift and genomic RNA mutations

The outer capsid protein VP2 contains the conformation-dependent antigenic host protective epitopes that elicit the production of neutralizing Ab (Cui *et al.*, 2003; Gómez *et al.*, 2013). The regions where the antigenic epitopes are located on VP2 have high nucleotide variability indicating its vulnerability to antigenic shift and drift (Jackwood & Stoute, 2013; He *et al.*, 2016). Molecular studies have shown that determinants for virulence, tissue tropism and pathogenic phenotype of the vvIBDV are controlled by some AA residues in the VP2 protein at positions 253, 279 and 284 (Aricibasi *et al.*, 2010; Jackwood & Stoute, 2013; Yamaguchi *et al.*, 1996). Also, data derived from reverse genetic studies indicated that a single mutation at amino acid position 253 or any other AA within the hypervariable region of VP2 is enough to alter IBDV virulence (Letzel *et al.*, 2007; O'Neill *et al.*, 2010; Lawal *et al.*, 2017). Thus, attenuated IBDV were shown to possess mutations at Q253H, A270E, D279N and A284T (Delgui *et al.*, 2013; Lawal *et al.*, 2017; Méndez *et al.*, 2017; Lawal *et al.*, 2018). Furthermore, vaccine escape mutants of Del-E variant viruses capable of infecting flocks immunized against other variant strains of IBDV (Jackwood & Sommer, 2005) were shown to have point mutations at position T222A in the first hydrophilic projection loop PBC and S254N in PDE loop, between the two major hydrophilic projections in VP2 (Jackwood & Sommer-Wagner, 2011). These two AAs are found at the tips of the VP2 loop structures and contribute to the antigenic drift of the variant IBDV strains (Jackwood & Sommer-Wagner, 2011). Moreover, a point mutation at AA D212N is commonly observed in the most recent vvIBDV isolates which may consequently influence the VP2 structure, antigenicity and virulence of the virus (Alkie & Rautenschlein, 2016). In addition, a glycine-serine mutation at G254D position (loop PDE) was observed in vvIBDV isolated from flocks vaccinated with caIBDV based vaccines (Negash *et al.*, 2012) hence this

mutation may possibly play a role in the frequently reported cases of vaccination failure (Jackwood & Sommer-Wagner, 2011). IBDV isolates with recombined AA sequences in the PBC and PHI loops and sequences in the minor PDE and PFG loops from both caIBDV and variant Del-E respectively were reported (Jackwood, 2012), while others have undergone intra-segmental recombination event within their VP1 encoding segment B, between two vvIBDV (Yip *et al.*, 2012). Similarly, a mutation at AA position A270E was observed in a vvIBDV isolate with unusual pathogenicity (Hoque *et al.*, 2001) which appeared to influence virulence as observed recently (Lawal *et al.*, 2017). Recent findings in the epidemiology of IBDV revealed the emergence of a distinct strain not known before, designated as distinct IBDV (dIBDV), having unique diagnostic AA sequences 272T, 289P, 290I, and 296F within the VP2 hypervariable region that are conserved and a 234P in the VP1, that is wide spread in South America, Europe and Asia (Hernández *et al.*, 2015; Tomás *et al.*, 2015) and which could not be classified into any of the previously known IBDV strains based on molecular signatures and pathogenicities. For VP1 protein, phylogenetic studies revealed more than one lineage of genome segment B of IBD viruses with high degree of conservation between vvIBDVs and non-vvIBDVs (Hon *et al.*, 2006). It has been shown that vvIBDV formed a single lineage of segment B genome while the non-vvIBDV formed at least four different genetic lineages containing caIBDV, vaIBDV and vaccine non-vvIBDV strains (Le Nouën *et al.*, 2006; Deng *et al.*, 2007). The segment B virulence associated sequences were recognized (Jackwood *et al.*, 2012) with reports of reduction in pathogenicity in IBDV reassortants possessing non-vvIBDV segment B and vvIBDV segment A (Deng *et al.*, 2007). Studies involving the virulence associated sequences revealed that a single AA exchange between an attenuated IBDV strain and vvIBDV strain at position 4 from valine to isoleucine (V4I) could result in decreased pathogenicity of the vvIBDV strain (Yu *et al.*, 2013). Similarly, recombination of the recently identified VP1 putative virulence markers T-D-N motifs in vvIBDVs with N-E-G or T-E-G found in attenuated strains at AA positions 145, 146, and 147 resulted in loss of virulence and vice versa (Jackwood *et al.*, 2012). Moreover, reassortant IBDVs containing genome segment A of vvIBDV and genome segment B of non-vvIBDV (Alfonso-Morales *et al.*, 2015) or serotype 2 strains with reduced pathogenicity exist in nature (Jackwood *et al.*, 2012; Soubies *et al.*, 2016). The current evidence of the role of VP1 in the virulence modulation of IBDV resulted in the renewed call for the involvement of both genome segments of the virus in molecular epidemiological studies before a conclusive epidemiological identity is assigned to an IBDV strain, prompting the identification and evaluation of a 430bp segment B marker framed between the N-terminal domain and partial F domain of the polymerase protein (Alfonso-Morales *et al.*, 2015) to be included in molecular epidemiology of IBDVs. Furthermore, because of the genetic diversity that is being witnessed based on IBDV VP2 molecular epidemiology, Michel & Jackwood (2017) suggested the adoption of a new IBDV classification into seven genogroups because the classical classification scheme traditionally used to classify the virus based on antigenic types and pathotypes is confusing. Some of the genogroups identified have global dispersion as is the case with the members of the genogroups 1 (caIBDV) and 3 (vvIBDV and its reassortants), while others such as members of the genogroup 2 (vaIBDV predominantly observed in the Americas), genogroup 4 (dIBDV predominantly found in South America) and genogroup 5 (IBDV predominantly found in Mexico believed to be recombinants of caIBDV and vaIBV) have geographic restrictions. Members of the genogroup 6 are viruses from the Middle East (Saudi Arabia) that showed 92 to 93% relatedness to ITA genotype in Italy and 94 to 95% relatedness to IBDVRF-5/94 Russian strain. Members of the genogroup 7 constitute mainly of viruses from Australia with some few from Russia (Michel & Jackwood, 2017). The basis for this classification is that some molecular signatures were observed apart from the 3 known AA sequences that are known to differentiate caIBDV, vaIBDV and vvIBDV from one another, with others such as those in genogroup three having AA mutation at position 222 from alanine to threonine, a location that is important in pathogenicity modulation since it is the first of the four surface loops of the hypervariable region of VP2 protein. Amazingly, members of the genogroup 2 have a shift from proline to threonine at the same AA position 222 (Figure 1). Interestingly, the genogroup classification correlates with the phylogenetic analysis of the various serotype 1 strains using complete polyprotein amino acid sequences with the caIBDVs, vvIBDVs and vaIBDVs clustering together (Figure 2). The predicted 3D structural model of the vvIBDV VP2 hypervariable region indicating the conformational epitopes where the neutralizing antibody binds to the IBDV to alter its infectivity in vivo (Arnold *et al.*, 2006; Bordoli *et al.*, 2009) is shown in Figure 3.

Immune Responses Against Infectious Bursal Disease Virus

Innate immunity

Immune responses to the IBDV infection had been studied extensively using the pathogenic strains from the serotype 1 viruses because the serotype 2 viruses thus far identified are found to be either non-pathogenic in turkeys, ducks and chickens (Berg, 2000; Jackwood & Saif, 1987) or slightly pathogenic (Yao *et al.*, 1998). Following the emergence of vaIBDV strains in the United States and vvIBDV strains in Europe, Asia and Africa, and their ability to cross high levels of protective antibody raised against the caIBDV strains in vaccinated flocks, the need to expound more on the current knowledge on the immune response to IBDV became apparent. The first isolation of variant strains were from chicken flocks with neutralizing Abs to serotype 1 strains of IBDV (Rodriguez-Chavez *et al.*, 2002) and both live attenuated and killed vaccines developed using variant strains conferred protection to challenged chickens with either classical or variant strains,

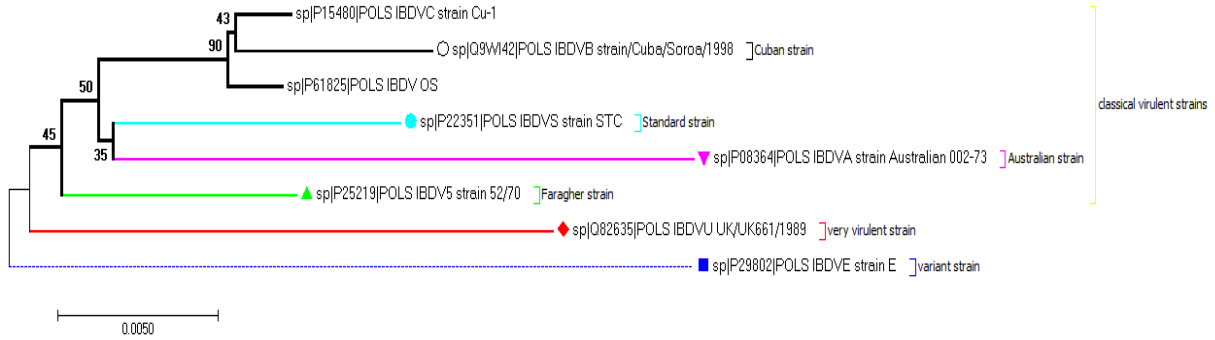

Figure 2: Evolutionary relationships of taxa

This figure showed the phylogenetic relationship that exists between various serotype 1 strains of IBDV: caIBDV represented by Soroa strain from Cuba, 002-73 strain from Australia, Cu-1 strain from Germany and standard STC & Faragher 52/70 strains from US; the vvIBDV represented by UK661 strain and the vaIBDV represented by strain E isolated from UK and US respectively. The evolutionary history was inferred using the Neighbor-Joining method (Saitou & Nei, 1987), with 2000 bootstrap (Felsenstein, 1985). The evolutionary distances were computed using the JTT matrix-based method (Jones *et al.*, 1992) and a gamma distribution pattern was used (shape parameter = 1). The analysis involved 8 amino acid sequences with a total of 1012 positions of the whole polyprotein sequence in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar *et al.*, 2016).

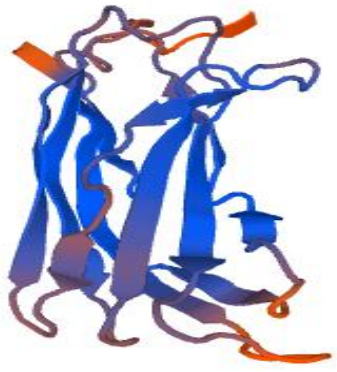

Figure 3: VP2 hypervariable region predicted 3D amino acid structural model of hvVP2 region of a vvIBDV showing the comformational-neutralizing antibody binding sites. The model was built using SWISS-Model freely available at ttps://swissmodel.expasy.org/interactive/mqyrNG/models/

whereas vaccines developed using classical IBDV strains partially protected or failed to protect against infection with the variant strains (Vakharia *et al.*, 1994) even though it was suggested that all the serotype 1 strains share minor antigens that evoke protective Ab production.

Gene expression studies revealed that bursal T cells and splenic macrophages are activated during acute IBD infection (Palmquist *et al.*, 2006; Khatri & Sharma, 2007). Early host responses are related with low levels of B cell specific genes as the bursal cells are depleted as well as up-regulation of genes involved with the activation of NK cells, macrophages and T cells (Smith *et al.*, 2015). These genes include those modulating interferon system, IL-6 and IL-18, IL-8 and MIP-1 and the genes modulating other arms of the innate immune system such as MD-1 and MD-2, complement components, heat shock proteins HSP70 and 47; and the inflammatory and pro-inflammatory response genes (Smith *et al.*, 2015).

Genetic regulations of innate immune response during IBDV infection

IBDV can be detected as early as 8–12 hours following oral infection in the mononuclear phagocytic cells of the intestine, which then carry the virus to the bursa for efficient viral replication in IgM bearing B-cells (Alkie & Rautenschlein, 2016). The infection activates nuclear factor (NF)-κB pathway and other intracellular signalling pathways (Khatri & Sharma, 2006). Large scale expression of proinflammatory mediators such as interleukin-6, IL-1β, and IL-18 as well as inducible nitric oxide synthase are induced by massive infiltration of macrophage into the bursa (Khatri *et al.*, 2005). Higher expression of interferon (IFN)-γ, chemokines (CXCLi2), acute phase proteins, complement components, and β-defensins in primary lymphoid organs were observed in IBDV experimental studies (Khatri & Sharma, 2007; Rasoli *et al.*, 2015). A 15-fold higher expression of NK lysine in experimental studies compared with uninoculated controls has been reported, suggesting a possible involvement of NK cells in the pathogenesis and innate immune response to IBDV infection (Smith *et al.*, 2015). Much is needed to be done to fully elucidate on the role of NK cells in IBDV infections.

An up-regulation of type I IFNs and the expression of viperin, myxovirus resistance 1 (Mx), and chicken zinc finger antiviral protein (ZAP) in BF and spleen tissues was reported following IBDV infection (Lee *et al.*, 2015; Yu *et al.*, 2020). Furthermore, IFN regulatory factor 7, janus kinases/signal transducer and activators of transcription (JAK/STAT), interferon induced protein with tetratricopeptide repeats 5, IFITM1, IFITM3, IFITM5, 2′-5′-oligoadenylate synthase, and dsRNA-dependent protein kinase were reported to be induced following IBDV infection (Smith *et al.*, 2015b). It has been shown that down-regulation of the IFN response by Gallus gallus microRNA-9 (gga-miR-9*) through IFN regulatory factor 2 may promote IBDV replication (Eldaghayes *et al.*, 2006; Ouyang *et al.*, 2015).

Active immunity

Active immunity against IBDV is stimulated when chickens are exposed to the wild type virus or when they are vaccinated with live attenuated or inactivated IBD vaccines. This response leads to production of specific neutralizing antibodies that can be measured using several serological tests such as agar gel precipitation (AGP), enzyme-linked immunosorbent assay (ELISA) and virus neutralization (VN) test. Antibody levels as high as 1:1000 was recorded post vaccination as measured by VN test (Saif, 2004). Cell mediated immunity was reported to be involved in protection against IBD infection when partial protection was observed following vaccination with recombinant fowlpox virus coding for IBDV VP2 protein in the absence of detectable neutralizing antibodies (Bayliss *et al.*, 1991).

Passive immunity

Maternal antibodies which last for about 2 to 3 weeks are passively transferred through the egg yolk from the hen to protect chicks against IBDV infections early on in life resulting in protection against its potential immunosuppression (Skeels *et al.*, 1979). Knowledge of the half-life of the maternally-derived antibody will help in determining the period of vulnerability of the progeny chicks to IBD virus infection. Experimental studies showed that young chicks are 40% protected from IBD infection with neutralizing antibody titers ranging from 1:100 to 1:600 but become highly susceptible to IBD infection when the titer decayed below 1:100, but for optimum vaccination with live attenuated virus, titer should fall below 1:64 (Skeels *et al.*, 1979). Failure to understand and monitor this period of MDA decay is a very big factor to vaccination failure leading to repeated outbreaks in flocks that were supposedly vaccinated against IBD and its attending economic losses to the farmers. Effective immunity was reported to be stimulated using inactivated killed IBD vaccines adjuvanated with oil emulsion in breeders conferring 4 to 5 weeks of protection in progeny chicks via MDA (Baxendale & Lutticken, 1981) while only 1 to 3 weeks protection was observed in progeny from breeders vaccinated with live vaccines. The disadvantage of this passive protection however, is that it can interfere with stimulation of an active immune response against IBDV if MDA half-life is not monitored (Lawal *et al.*, 2017).

Host Pattern Recognition Receptors in the IBDV Immune Response

Invading pathogens first encounter the innate immune response as the first-line of defense in their host (Alkie & Rautenschlein, 2016). The receptors that control innate immunity are called pattern recognition receptors (PRRs), which scan the surfaces of pathogens for recognition of pathogen-associated molecular patterns (PAMPs) for generation of effector molecules (Doyle & O'Neill, 2006). There are about ten Toll-like receptors (TLRs) that have been identified in chickens through which IBDV interact with the host (Kogut *et al.*, 2005). TLR3 and TLR21 are upregulated when mucosal infection of chickens with IBDV occurs (Lee *et al.*, 2015; Smith *et al.*, 2015) while in some cases, TLR7 differential regulation occurs

following IBDV mucosal infection (Rauf *et al.*, 2011) suggesting involvement of TLRs in recognition of IBDV for the induction of innate immunity. Other suggested intracellular PRR for IBDV include the chicken melanoma differentiation-associated gene (MDA5) (Lee *et al.*, 2014) which exhibits increased expression in the BF during early onset of mucosal IBDV infection (Smith *et al.*, 2015).

Adaptive Immune Responses and Mechanisms to Prevent IBDV Infection

The IBD virus employs strategies to escape the innate immune responses through impairment of the function of the antigen presenting cells or the interference with the IFN production pathway (Ye *et al.*, 2014; Yu *et al.*, 2020). To effectively combat viral infections, the host depends on specific adaptive immune responses to neutralize invading pathogens (Alkie & Rautenschlein, 2016). Following IBDV natural infection or vaccination, neutralizing Abs are produced against VP2 conformational epitopes few days after exposure, effectively neutralizing the virus providing adequate protection against the virus or other antigenetically related viruses (Fahey *et al.*, 1989).

Humoral immunity

The major effects of IBD on infected chickens is the destructive depletion of follicular lymphoid cells (Ley *et al.*, 1983) resulting in a significant reduction in the chicken's humoral responses to infections. IBDV infected chickens showed a massive decrease in the population of IgM bearing B cells in the bursa of Fabricius especially immature ones (Sivanandan & Maheswaran, 1980). This occurs in the bursal medulla and cortex, thymic medulla and peripheral blood (Ramm *et al.*, 1991) with some degree of losses of the surface antigens of the bursal epithelial cells but T cells are refractory to infection (Saif, 1991). Bursal infiltration with CD4+ and CD8+ T cells was reported to begin by 1 day post infection (dpi) with T cells reaching 65% of the cell population 7 dpi (Sivanandan & Maheswaran, 1980).

Cell mediated immunity

The cell-mediated immune response is demonstrated in IBD virus infection by an increase in the mRNA transcripts of the pro-inflammatory cytokines. This increase in cytokines such as IL-1b, IL-6, CXCLi2, and IFN-c was observed following in vivo infection of chickens with various strains of IBDV in conjunction with concurrent suppression of transforming growth factor-b4 (Eldeghayes *et al.*, 2006). In one study, positive correlation was reported between the up-regulation of IFN-c and IL-12a but not IL-12b or IL-18 during IBDV infection. Additionally, an increase in the level of IL-18 mRNA in splenic macrophages was observed five days post IBDV (Palmquist *et al.*, 2006). Pro-inflammatory cytokines such as IL-1b, IL-6 and inducible nitric oxide synthase have their mRNA transcripts up-regulated, so also are the transcriptional levels of type I, II, and III IFN as well as IL-18, IL-4, and IL-13 as observed in IBDV in vivo challenge studies (Mahgoub, 2010).

Immunosuppression

The immunodepressive effects of IBD virus was observed and reported by Allan *et al.* (1972) and Faragher *et al.* (1974). Chickens infected with the virus at 1 day of age had the highest depression of the humoral response to Newcastle disease virus with the suppression being moderate at 7 days and negligible when infected at 14 and 21 days of age (Faragher *et al.*, 1974). Response to vaccines against other diseases were also reported to be significantly low as reported by Hirai *et al.* (1974) with increased susceptibility of infected chickens to wide range of diseases such as inclusion body hepatitis (Fadly *et al.*, 1976), coccidiosis (Anderson *et al.*, 1977), Marek's disease (Cho, 1970), hemorrhagic-aplastic anaemia (Yuasa *et al.*, 1980), infectious laryngotracheitis (Rosenberger & Gelb, 1978), infectious bronchitis (Pejkovski *et al.*, 1979), salmonellosis and colibacillosis (Wyeth, 1975) among others. It is interesting, however, to note that there is normal humoral response to IBDV infection even though there is immunosuppression against other agents even in day old chicks (Skeeles *et al.*, 1979).

The effect of the virus on the cellular arm of the immunity appears to be transient and occult than its effect on humoral responses. This was evident in the earlier reports of the association of IBDV virus with respect to delay in skin graft rejection in IBDV infections (Panigrahy *et al.*, 1977). This was however, different from the findings of Giombrone *et al.* (1977) or Hudson *et al.* (1975) who observed no effect in early IBDV infections on skin graft rejection or tuberculin-delayed hypersensitivity reaction. Sivanandan and Maheswaran (1980; 1981) reported the maximum IBDV immunosuppressive effect on cellular arm of the immunity to be 6 weeks post infection (pi) with the lymphoblast transformation assay while another study reported extensive CMI depression following infection from 3 dpi up to 4 weeks pi in poults in response to concanavalin A exposure (Nusbaum *et al.*, 1988).

Current Vaccines Used in the Control of IBD

The various vaccines utilized for the control of IBD in poultry are presented below and their advantages, disadvantages and possible ways of improvement have been summarized in Table 1.

Live attenuated vaccines

Conventionally, attenuated IBDV vaccines are divided into mild, intermediate, or intermediate plus (hot) vaccines and when applied in drinking water can induce solid immunity (van den Berg *et al.*, 2000). However, their various degrees of immunosuppressive effects, reversion to virulence, and potential reservoir for genetic reassortment are major safety concerns, thus, warranting the search for novel and safe vaccines (Rautenschlein *et al.*, 2005; He *et al.*, 2014). In addition, mild vaccines are ineffective when there is high level of maternal antibodies or vvIBDV infection (Muller *et al.*, 2012). Intermediate and hot vaccines are more effective even in the presence of higher level of maternal antibodies, but may adversely cause immunosuppression due to lymphocyte depletion and bursal atrophy, depending on the severity of the damage (Rautenschlein *et al.*, 2005; Jackwood *et al.*, 2008). This may hinder their efficacy on chickens infected with vvIBDV strains (Rautenschlein *et al.*, 2005; Muller *et al.*, 2012). Despite the effectiveness of live attenuated vaccines, interference with maternal antibody at the early weeks of chicken life remains a major concern (Block *et al.*, 2007).

Inactivated killed vaccines

Killed vaccines contained either inactivated viruses, viral subunits or recombinant viral antigens and are less immunogenic compared to live attenuated vaccines unless they are administered repeatedly with adjuvants or as a booster dose after live attenuated vaccines administration (Lawal *et al.*, 2018). Killed vaccines are labor-intensive and costly in terms of administration thus, limiting their usage only in birds of high economic value such as breeders, where point of lay vaccination passively confers immunity to their progenies in ovo (Alkie & Rautenschlein, 2016). Nevertheless, their administration in 1-to-10-day old chicks in heavily IBDV contaminated environments has been reported (Wyeth & Chettle, 1990). Killed IBD vaccines are administered via the subcutaneous route as water-in-oil emulsion and they induced strong T-cell activation and inflammatory responses (Rautenschlein *et al.*, 2002) but must have high antigenic content for them to effectively protect progeny chickens from vvIBDV strains (Rosenberger *et al.*, 1987; Muller *et al.*, 1992).

Genetically engineered vaccines

Reverse genetics have been employed to modify IBDV and thus making it attenuated for live vaccine agents (Mundt & Vakharia, 1996) since the successful establishment of a reverse genetic system for the entire nucleotide sequences of the IBDV RNA genome segments (Mundt & Muller,1995). As the viral capsid protein, VP2 carries immunodominant epitopes responsible for the induction of a protective humoral immune response (Becht *et al.*, 1988; Fahey *et al.*, 1989; Muller *et al.*, 1992), the gene encoding the polyprotein or mature VP2, or immunogenic/neutralizing domains of VP2 are targeted to produce new generation candidate vaccines. In generating this type of vaccine, the VP2 gene is genetically manipulated to insert the aas Q253H, D279N and A283T mutations responsible for the virus attenuation (Islam *et al.*, 2001a; van Loon *et al.*, 2002; Raue *et al.*, 2004; Noor, 2009). Unfortunately, the mutated viruses easily revert to virulence following few passages in chickens (Raue *et al.*, 2004; Noor, 2009). Some studies showed that generated reassortants of serotype 1 and 2 IBDV induced high levels of serotype specific neutralizing antibodies (Oberlander, 2004; Zierenberg *et al.*, 2004) but they can also revert to virulence. IBDV-VP2 subunit vaccines produced in yeast and *Escherichia coli* expression systems have been licensed for commercial use (Vakharia *et al.*, 1994; Pitcovski *et al.*, 2003; Rong *et al.*, 2007), in addition to the development of virus-like particles (VLP) of IBDV (Jackwood, 2013) using genetic engineering, but they all have the same limitations with killed inactivated vaccines. However, these vaccines can be used as DIVA technology to differentiate naturally infected and vaccinated flock (Muller *et al.*, 2012). IBDV DNA vaccine has been developed (Fodor *et al.*, 1999) encoding polyprotein and offer more protection than the vaccines made from cDNA encoding VP2 alone (Li *et al.*, 2003). Moreover, the cDNA vaccine was reported to be more effective if the polyprotein sequences were recombined with either interleukin-2 or interleukin-6 DNA sequences (Li *et al.*, 2004; Sun *et al.*, 2005). DNA vaccines may elicit cell-mediated immunity including memory T-cell responses (Chang *et al.*, 2001; Hsieh *et al.*, 2010) contributing to the protective efficacy of the vaccine. They can be used to immunize eggs and day-old chicks both with booster doses of either killed or recombinant vaccines (Haygreen *et al.*, 2006; Hsieh *et al.*, 2007). They are however, expensive to develop but have the advantage of being stable and lack the risks of reversion to virulence or being a possible vessel of genetic reassortment.

Table 1: Summary of IBD vaccine type, their advantages and disadvantages and possible way forward for improvement

Vaccine type	Advantages	Disadvantages	Possible improvement
Live attenuated IBD vaccines	Long lasting immunity, induces mucosal immunity, single dose requirement.	MDA interference, reversion to virulence, genomic recombination with wild isolate leading to emergence of new strain with enhanced virulence.	Proper non-reversible attenuation, development of IBDV permissive cell lines that can readily adapt and attenuate vvIBDV with generation of high viral titre and are compatible with mass production technology such as bioreactors.
Killed/inactivated IBD vaccines	Safe- no reversion to virulence, cheap to develop, no cold chain requirement.	Tissue damage due to vaccine reaction, multiple booster dosing is required, do not induce mucosal immunity.	Development of good adjuvants with potent induction of inflammatory response.
Live viral vector IBD vaccines	Protection against multiple organisms, safety-no reversion to virulence, expensive to develop, sustained antigen release through integration of the viral sequence of interest into the host chromosomes by viral vectors that establish persistent infection in the vaccinated host.	Pre-existing immunity to the viral vector, interference by MDA, defective post translational modification can increase or decrease the antigenicity of conformation dependent epitopes, replication interference by other vaccines made from the vector given at the same time.	Improvement on the coding capacity of the vectors by removing redundant replication-independent non-coding sequences, improvement in current molecular based-adjuvants development.
Subunit IBD vaccines	Can be used as a DIVA system using VP2 alone as the subunit vaccine agent to differentiate vaccinated from infected flocks, since the internal capsid protein (VP3) induces the production of anti-VP3 antibodies only detected in infected flocks.	Parenteral administration is need, induces tissue damage due to vaccine reaction, multiple booster dosing is required.	Polyepitope based vaccine development, novel adjuvants development, new generation delivery system such as nanoparticles, liposomes and immune stimulating complex (ISCOM) as peptide delivery vehicle
DNA IBD vaccines	Naked DNA can be easily used, encoding the target gene, into host cells. No MDA interference, promotes the induction both humoral and cell mediated response following antigen expression in the cells.	There may be difficulties in secreting or translocating the expressed viral protein from the cell. Booster vaccination may b required especially in *in ovo* vaccination.	Conjugating the DNA (VP2-VP4-VP3) sequences with sequences of interleukins (IL-1 and IL- 6) as adjuvants to improve efficacy.
Immune complex IBD vaccines	Effective for *in ovo* and subcutaneous administration in eggs or day-old chicks, causes low level of bursal and splenic lymphocyte depletion and circumvent high levels MDA.	Delay in virus detection following administration may interfere with DIVA strategy, neutralization of the live virus component by the antibody in the complex, thereby reducing sustained antigen stimulation.	

Viral vector vaccines for IBDV have been reported and could be used to vaccinate embryo or day-old chicks (Le Gros *et al.*, 2009) and were effective even in the presence of high MDA levels (Muller *et al.*, 2012) by protecting chickens against both vvIBDV and vIBDV challenge (Perozo *et al.*, 2009; Muller *et al.*, 2012). The viruses utilized as vectors include fowl pox virus (Bayliss *et al.*, 1991), Marek's disease virus (MDV) (Tsukamoto *et al.*, 1999, 2000, 2002), fowl adenovirus (FAdv) (Francois *et al.*, 2004), Semliki Forest virus (SFV) (Phenix *et al.*, 2001) and herpesvirus of turkey (HVT) (Darteil *et al.*, 1995). Some of the viral-vectored vaccines such as the herpesvirus of turkeys-IBD vaccine were licensed for in ovo and post hatch vaccination of broilers and layers in various countries (Bublot *et al.*, 2007; Le Gros *et al.*, 2009). They induced strong systemic neutralizing and/or mucosal antibodies. They however, are limited by a possible vaccine interference with other HVT vaccines given at the same time, which may affect vaccine efficacy by reducing their replication in host tissues.

Challenges Associated with IBD Vaccine Development

With over six decades of IBD emergence, several efforts have been put towards the control and prevention of the disease world over, but still the malady remains one of the most serious threats to the global poultry industry with no immediate hope of eradication. Just like many other infectious diseases, the main method of controlling IBD infection is through an efficient vaccination program, yet there are reports of outbreaks even in flocks with up-to-date vaccination records. Several limitations and challenges have been identified to be associated with IBD vaccines currently available in the market and those still under development as discussed below:

Live vaccines: Vaccination of breeder flocks with live IBD vaccines is done to confer maternal immunity to their offspring (Muller *et al.*, 2012). Antibodies generated in this way offer protection to the chicks from early infections with IBDV for about 1–3 weeks which may be extended for 4 – 5 weeks with administration of oil-adjuvanted vaccines (Baxendale & Lutticken, 1981). This poses a major obstacle for vaccination of young chickens with live attenuated vaccines due to MDA interference, which makes determination of the optimal vaccination time critical to the success of vaccination with a live attenuated vaccine (Muller *et al.*, 2012). The choice of vaccine type and its level of attenuation is another critical factor to consider when vaccinating with live vaccines, as intermediate and intermediate plus (hot) IBD vaccines can cause various degrees of bursal damage and lymphoid cell depletion resulting in immunosupression and a possible transient increased susceptibility to other pathogens. Further, they may not offer full protection against variant or vvIBDV strains (Rautenschlein *et al.*, 2005). Moreover, vaccines that have poorly attenuated IBD virus have the risk of reversion to virulence after few replications in the bursa of Fabricius, increased chances of horizontal transmission to susceptible chickens and possible vaccine-induced reaction that may cause decreased productivity or disease. In addition, there is a requirement for cold chain maintenance during transit and storage for viability of the virus to be preserved (Muller *et al.*, 2012). The advantage of these vaccines is that they can cross high levels of maternal antibody to localize in lymphoid organs such as bursa, thymus and spleen and persist for 2 weeks, allowing an active humoral immune response to be mounted against them following the fall in the maternal antibody titre (Muller *et al.*, 2012).

Killed vaccines: Majority of killed IBD vaccines are water-in-oil emulsions, usually combining several antigens and were reported to be able to elicit cell mediate immunity and strong inflammatory responses in vaccinated chickens (Rautenschlein *et al.*, 2002). Their limitations however, are they must have large amount of antigen in them before they can induce protective immunity in breeders which can be extended to their progeny through MDA to protect them from infection by variant or vvIBDV strains (Rosenberger *et al.*, 1987; Muller *et al.*, 1992); they must also be given in large multiple doses in a prime-booster regimen for them to be effective being non-replicating entities. They frequently induce massive tissue reaction (vaccine reaction) due to the inflammatory response evoked by the adjuvants incorporated in them; they are more costly; may cause vaccine failure and disease outbreak if poorly inactivated and do not induce mucosal immunity (Muller *et al.*, 2012).

Genetically engineered vaccines

The advances in genetic engineering technology made it possible to produce IBD vaccines since the establishment of reverse genetic system for both genome segments of IBDV. Attenuated IBDVs are generated from parent vvIBDV by introducing specific mutations using site-directed mutagenesis of nucleotide sequences coding for specific amino acids in VP2 protein (Islam *et al.*, 2001b; van Loon *et al.*, 2002; Raue *et al.*, 2004; Noor, 2009). However,

reversion to virulence was frequently observed when the engineered virus replicates few times in vaccinated chickens (Raue *et al.*, 2004; Noor, 2009). This means for the genetically engineered viruses to be effectively used safely as vaccine, there may be the need to introduce quite a number of nucleotide mutations distributed throughout both genome segments of IBDV (Meeusen *et al.*, 2007; Noor, 2009).

Recent Advances in Vaccine Delivery and Adjuvant Technology

Vaccine delivery

Recently, nanotechnology has been used to develop vaccine delivery systems from biodegradable polymers such as chitosan and polylactic-co-glycolic acid (PLGA) to increase the efficacy of poultry vaccines, especially where mucosal immunity is concerned (Negash *et al.*, 2013). Vaccines delivered using this system are encapsulated within the nanoparticles and therefore, protected from enzymatic degradation. This way, there is sustained antigen release that will elicit long lasting immunity, thus eliminating the need for frequent applications of costly booster doses (Jones *et al.*, 1998). Furthermore, professional antigen presenting cells can be readily targeted for antigen delivery to facilitate recognition, processing and presentation (Kazzaz *et al.*, 2006) with significant enhancement of mucosal immunity. Negash *et al.* (2013) encapsulated plasmid containing IBDV DNA and chicken IL-2 in PLGA microparticles (MPs) which when used for immunization effectively protected immunized chickens against challenge, with measurable cell mediated immunity (CMI). Success has also been reported in mucosal IBD vaccine delivery using transformed bacteria such as *Escherichia coli* and Salmonella species (Mahmood *et al.*, 2007) to orally deliver recombinant plasmid encoding polyprotein gene with 73% protection recorded. Again, protective potential of recombinant DNA vaccine containing VP2 gene of a vvIBDV adjuvanted with CpG oligonucleotide in chickens was reported (Mahmood *et al.*, 2006). Nanoparticle delivery system therefore offers unlimited opportunities that will enhance the effectiveness of adaptive and innate immune responses following IBD vaccination.

New generation vaccine adjuvants

The major limitation with synthetic or recombinant antigens used in new generation vaccinology is their less immunogenic nature than live attenuated or inactivated vaccines (Hsieh *et al.*, 2010; Chen *et al.*, 2011) necessitating the need for incorporation of compounds that improve the specific immune response against co-administered antigens (Hulse & Romero, 2004; Mahmood *et al.*, 2006). Research showed that subunit and DNA vaccines so far generated against IBDV offer partial protection when they are used alone without any adjuvants. The incorporation of interleukin sequences such as IL-2, IL-4, IL-6, IL-12, IL-18 and heat shock protein 70 (HSP70) to the VP2 or polyprotein subunit or DNA vaccines has proven to significantly enhance the efficacy of these vaccines in terms of immune response and protection offered (Kumar *et al.*, 2009; Su *et al.*, 2011; Muller *et al.*, 2012; Li *et al.*, 2013; Maity *et al.*, 2015). However, further research is needed in the field of adjuvants technology to further enhance the immunomodulation of the current ones and identify new potential adjuvant since poor responses had been recorded with recombinant IL-1 β and IFNs when combined with a killed IBD vaccine (Schijns *et al.*, 2000). Other potential adjuvants identified and tested include CpG oligodeoxynucleotides (Mahmood *et al.*, 2006; Negash *et al.*, 2013), chicken beta-defensin-1, (Zhang *et al.*, 2010) and porcine lactoferrin (Hung *et al.*, 2010), with various degrees of success (Alkie & Raustenchlein, 2016).

Reverse genetics and vaccinology

The introduction of vaccines targeting reassortant subsets of circulating IBDVs may be necessary in future IBDV control strategies. Different reverse genetic systems for IBDV have been described, which can be used for better characterization and vaccine development (Mundt & Vakharia 1996; Silva *et al.*, 2014). A modified IBDV that contained a 3′ RNA sequence generated by cis-acting hepatitis delta virus ribozyme was less pathogenic to the BF compared with a cell line-adapted variant E IBDV strain, but induces higher Ab responses as early as day 7 post infection (Mosley *et al.*, 2013). The risk of reversion to virulence of the genetically modified viruses may however exist (Raue *et al.*, 2004).

Conclusion

Vaccinology research has several barriers that may restrict the needed speed it requires. Lack of investment incentives especially in developing countries; the antigenic drift and shift leading to generation of new reassortant viruses with antigenic diversity within the serotype 1 viruses allowing them to escape host immune responses; stringent regulations for vaccine registration and the public perception of the consumption of food products

derived from animals vaccinated using reverse genetic technologies are among the few identified obstacles in IBD vaccine research and development. Despite these hurdles and many more, significant progress and advancement in IBD vaccinology had been achieved since its emergence that permits an effective control alongside strict biosecurity. The emergence of variant IBDV, vvIBDV and dIBDV within the poultry population of the world promoted the search for more effective vaccines to control the disease. Live attenuated vaccines have been successfully used but are limited by their risk of reversion to virulence and interference with MDA with the exception of hot vaccines, which can cross high levels of MDA albeit with damage to the bursa, lymphoid cell depletion and possible immunosuppression. Live viral vectored IBD vaccines are effective but are equally interfered with by MDA and/or other vaccines made from the same vector when co-administered together. Naked DNA vaccination via mucosa showed variable efficacy in terms of protection offered, with DNA vaccines co-administered with genetic adjuvants being superior to those containing only target DNA. Efforts should therefore be on the search for identification and development of new delivery systems that will allow precision vaccination powerful enough to stimulate both innate and adaptive (humoral and cellular) immune responses. It has been demonstrated that autophagy via the AKR-thymoma-mechanistic target of rapamycin (AKT-MTOR) dependent pathway can be induced by VP2-avibirnavirus receptor interactions (Hu *et al.*, 2015) and the potential use of this phenomenon in the future IBD control has been highlighted (Alkie & Raustenchlein, 2016). The use of nanoparticles, liposomes, ISCOMs and virosomes for IBD vaccine delivery has been explored with success, but efforts are needed to address stability and possible toxic effects on the chicken and their human consumers (Petrovsky & Aguilar, 2004).

Conflict of Interest

The authors declare that there is no conflict of interest.

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