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REVIEW

Infectious bursal disease virus in chickens: prevalence, impact, and management strategies

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Abstract: Infectious bursal disease (IBD), also known as Gumboro disease, is a highly contagious, immunosuppressive disease of young chickens. Although first observed about 60 years ago, to date, the disease is responsible for major economic losses in the poultry industry worldwide. IBD virus (IBDV), a double-stranded RNA virus, exists as two serotypes with only serotype 1 causing the disease in young chickens. The virus infects the bursa of Fabricius of particularly the actively dividing and differentiating lymphocytes of the B-cells lineage of immature chickens, resulting in morbidity, mortality, and immunosuppression. Immunosuppression enhances the susceptibility of chickens to other infections and interferes with vaccination against other diseases. Immunization is the most important measure to control IBD; however, rampant usage of live vaccines has resulted in the evolution of new strains. Although the immunosuppression caused by IBDV is more directed toward the B lymphocytes, the protective immunity in birds depends on inducement of both humoral and cell-mediated immune responses. The interference with the inactivated vaccine induced maternally derived antibodies in young chicks has become a hurdle in controlling the disease, thus necessitating the development of newer vaccines with improved efficacy. The present review illustrates the overall dynamics of the virus and the disease, and the recent developments in the field of virus diagnosis and vaccine research.

Keywords: infectious bursal disease, epidemiology, pathogenesis, diagnosis, vaccines

Introduction

Infectious bursal disease (IBD), also known as Gumboro disease is a highly contagious and immunosuppressive disease of young chickens caused by IBD virus (IBDV) which is responsible for major economic losses in the poultry industry worldwide. IBDV is a double-stranded RNA virus belongs to the genus *Avibirnavirus* of the family *Birnaviridae*.[1] The genome of the IBDV is bi-segmented and divided into segment A and B. The larger openreading frame 1 (ORF1) of segment A encodes for a 110 kDa polyprotein which auto-catalytically splices into viral proteins VP2 (48 kDa), VP3 (33–35 kDa) and VP4 (24 kDa). IBDV has two recognized serotypes, namely serotypes 1 and 2 and both serotypes of the virus can naturally infect chicken, turkey, duck, guinea fowl, ostriches; the pathogenicity being reported only in chicken by serotype 1. The "re-emergence" of IBDV as antigenic variants and very virulent strains (vvIBDV) had been the reason for significant losses and high mortality in chickens, and the virus is continuously evolving in the field with changes in antigenicity and virulence. VP2 is the major host-protective capsid protein of the virus that carries the immunogenic determinants and is able to elicit neutralizing antibodies. Recently, IBDV has been

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classified into seven genogroups based on the marked changes in the amino acids in the hypervariable region of the capsid protein VP2 (hVP2) among the different groups, wherein genogroup 1 is distributed globally. Currently, the disease is controlled by live attenuated or inactivated IBDV vaccine, but the live vaccines can revert back to virulence and the traditional vaccines may not give full protection against vvIBDV strain. The inactivated or killed vaccines are usually given to birds in pre-laying stage to induce higher levels of antibody production for at least 2 weeks. However, with the emergence of antigenic variants and very virulent IBDV (vvIBDV), chickens are not fully protected by conventional IBD vaccines. Thus, new generation vaccines are produced with the advantage of their overcoming the interference with maternally derived antibodies (MDA), besides safety, ease of production and stability.

IBDV genome organization

The IBDV genome consists of two segments of double-stranded RNA which are packaged in a non-enveloped icosahedral shell having 32 capsomers, 60 nm in diameter. The structure of the virus is based on a T=13 lattice and the capsid subunits are predominantly trimer clustered.[2] The larger segment A encodes for a polyprotein (pVP2–VP4–VP3) which is 3,261 nucleotides long and encodes a 110 kDa precursor protein in a single large ORF. In the VP2 protein, a precursor–product relationship exists as only the larger protein (pVP2, 48 kDa) can be demonstrated in infected cells, and further proteolytic cleavage transforms the precursor into the VP2 protein, present in the complete virus particle.[3] This polyprotein can be digested to pVP2 (1–512 amino acids), VP4 (513–791 amino acids) and VP3 (792–1,012 amino acids) proteins by autoproteolysis of VP4 to yield mature VP2–VP4 proteins prior to viral assembly. VP2 being the major host-protective capsid antigen contains at least two epitopes for neutralizing antibodies that protect the susceptible host from vIBDV and is the determinant for cell tropism, tissue culture adaptation and pathogenic phenotype of IBDV.[4] VP2 protein has three major domains, namely the base, shell and projection domains. The conserved amino acids form the base and the shell domains whereas the projection domain is formed by a hypervariable region of VP2 spanning amino acids 206–350.[5] VP3 (32 kDa) is a group-specific immunogenic protein of IBDV, has cross-reactivity with both serotypes 1 and 2 and following infection the earliest appearing antibodies are directed toward VP3.[6,7] Segment A also encodes a 17 kDa non-structural protein VP5, with a partially overlapping ORF.[8] VP5 is a class 2 membrane protein with a cytoplasmic N-terminal and extracellular C-terminal domain and plays an important role in pathogenesis. This protein is highly basic, cysteine-rich and semi-conserved among all the serotypes of IBDV.[8] VP5 accumulates in the cell membrane resulting in cell disruption and virion release. The smaller segment B encodes VP1 (97 kDa), an RNA-dependent RNA polymerase and it exists both as a free polypeptide and as a genome-linked protein. It plays a key role in the encapsidation of the viral particles and interacts with VP3 to form the VP1-VP3 complex that gives the structural integrity for the viral particles.[9]

Prevalence

Infectious bursal disease (IBD) has been a serious threat to the poultry industry. The "re-emergence" of IBDV as antigenic variants and hypervirulent strains had been the reason for significant losses and high mortality. However, factors like the dose and virulence of the strain, age and breed of the birds and the presence or absence of passive immunity may be linked with mortality. The disease leads to immunosuppression and the infected birds become susceptible to other viruses, bacteria or parasites. Moreover, the increased usage of antibiotics against secondary infections may also lead to a growing public health concern.

Distribution of IBDV

IBDV termed as avian nephrosis or "classic IBDV" was first reported from Gumboro in Delaware, USA in the year 1962[10] and hence the name of the disease was originated besides "IBD" or "infectious bursitis." The disease has spread to most parts of the USA between 1960 and 1964, and affected Europe in between 1962 and 1971.[11] IBDV strains based on virus neutralization assay are classified into serotype 1 and serotype 2, While serotype 1 viruses are pathogenic to chickens and based upon the mortality and bursal lesions, the virus can be categorized into attenuated (atIBDV), classical virulent (cvIBDV), antigenic variant (avIBDV) and very virulent (vvIBDV) subtypes.[12] Serotype 2 viruses are isolated from turkeys and are avirulent to chickens. The vvIBDV strains that were more virulent than classical strains causing mortality rates of 90% were detected in Holland in 1986. The virus strain DV86 then had spread to the UK in 1988 and was detected in Japan and Belgium.[13] Since then the acute form of the disease has spread to most of the countries including all of Asia, Central Europe and Russia, the Middle East, South

America[14] with only Australia, New Zealand, Canada and the USA remaining free till 2008. However, there are significant differences between the African, European and Asian vvIBDV strains, suggestive of an independent evolution.[14] However, the variant strain of serotype 1 virus that emerged in the USA in 1985 causes no mortality but induces rapid and severe bursal atrophy. The live-attenuated vaccines developed against classical strain fail to respond against the variant strains. Moreover, in the USA, the monoclonal antibodies developed against VP2 fail to react with one-third of the 300 IBDV field isolates that proves the rapidity with which the virus had evolved. Based on a recent study, it is proposed that, worldwide, about 60–76% of IBDV isolates are of vvIBDV genotype.[15] Rampant vaccination without genotype matching resulted in genetic diversification of circulating viruses through reassortment that acquired segment A from very virulent IBDV and segment B from classical atIBDV D78 strain in Poland.[16] The chimeric virus caused 80% mortality in Specific Pathogen Free chickens along with acute form of bursal lesions.

Economic impact

The economic impact of IBD on the poultry industry is difficult to assess due to the complex nature of losses associated with the disease. IBDV infection-induced immunodeficiency in chickens makes the flock susceptible to other viral, bacterial, and parasitic infections, thus resulting in indirect losses. Being resistant to most of the disinfectants and environmental factors, the poultry house remains contaminated with IBDV that persists on the premises and tends to reappear in subsequent flocks.

The acute or chronic IBD in the flock reduces the efficiency of production and net profitability in terms of feed conversion ratio. The avIBDV strains prevalent in Saskatchewan farms probably attributed to substantial economic losses of about 3.9 million kg per year to broiler meat industry.[17]

Molecular epidemiology and phylogeny analysis of IBDV

Vaccination is the most important measure to control IBD; however, extensive usage of live vaccines has resulted in the evolution of new strains. Despite the regular vaccination schedule, there are still reports of IBDV outbreaks around the globe. By closely studying the evolution of IBDV, it is possible to find the cause of these outbreaks. The major capsid protein VP2 plays a pivotal role in antigenic variation by which the virus can escape neutralizing antibodies. Most of the amino acid (aa) changes among the antigenically different IBDVs are clustered in the hypervariable region of VP2 (hVP2). Thus, this hypervariable region of VP2 is the obvious target for IBDV detection, evolution and pathogenic variation. The hVP2 region has two major hydrophilic domains, namely major hydrophilic peak A (aa 212–224) and peak B (aa 314–325) that form hairpin loops P_{BC} (aa 219–224) and P_{HI} (aa 316–324), respectively. The minor hydrophilic peak 1 (aa 248–254) and peak 2 (aa 279–290) of hVP2 form loops P_{DE} (aa 249–254) and P_{FG} (aa 279–284), respectively.[18] Either single or combined mutations in hVP2 region affect the virulence pattern of the virus. Recent IBDV field outbreaks from various geographic locations revealed amino acid exchanges at minor hydrophilic peak domain of hVP2.[19] Mutations at the variable domains between amino acids position 206 and 350, where the neutralizing antibody binds, results in immune evasion of the virus. The substitution of the amino acids at positions 253 (Q253H), 279 (D279N) and 284 (A284T) on the VP2 domain of vvIBDV resulted in loss of virulence of the virus. But a single point mutation in the 253 (H253Q/N) or 249 (R249Q), however, drastically increased the virulence of an attenuated IBDV strain. The mutation at the amino acid position 254 with serine in place of glycine (loop P_{DE})[20] results in vaccination failure. Although sequencing of the whole IBDV genome is useful for phylogenetic characterization, it is impractical to replicate in large number of isolates. Recently, the virus has been classified into seven genogroups. Genogroup 1 generally comprises the classical IBDV and is present worldwide, genogroup 2 primarily comprises the antigenic variants predominant in the USA and genogroup 3 comprises predominantly of the vvIBDV pathotype or few are vvIBDV reassortants, distributed worldwide. An antigenic drift at aa position 222, (A222T) the genetic hallmark, has been observed in the population of genogroup 3 due to selective antigenic pressure with vaccination. Moreover, the genogroup 3 reassortants have a different segment B and do not have the typical amino acids found in the vvIBDV. Most of the virulent viruses of Indian origin also belong to genogroup 3 (Figure 1). However, the viral isolates that did not clearly fit into any of the three major genogroups were classified separately. The genogroup classification method classified these viruses into four new genogroups 4–7. A genogroup 4 virus is characterized by 222S, 272T, 289P, 290I and 296F and is distributed worldwide but was most commonly isolated from Latin America. Genogroup 5 viral strains included Mexican recombinant classical and variant viruses with amino acid changes in both P_{DE} and P_{HI}. These viruses have variant-

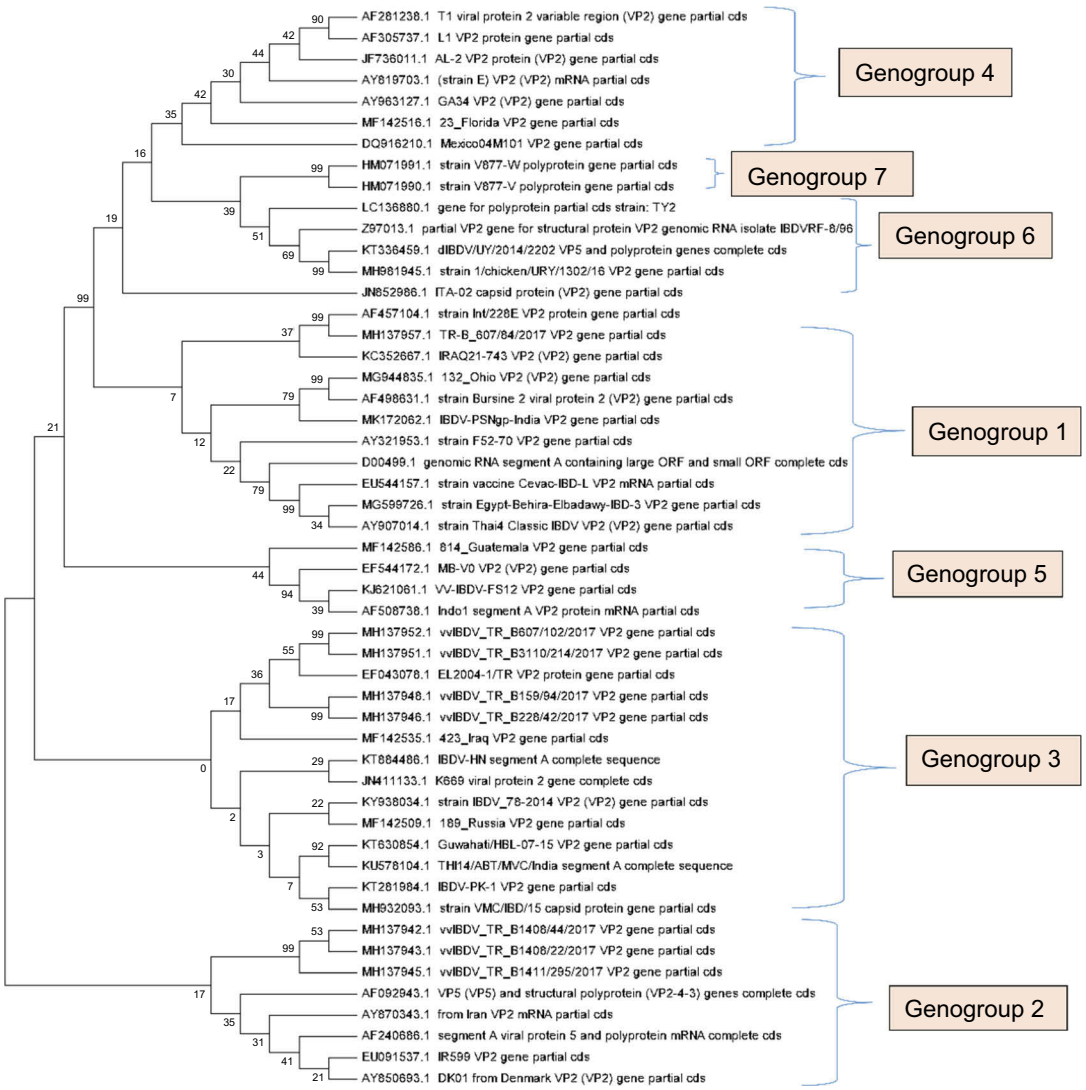

Figure 1 Phylogenetic analysis of the nucleotide sequences of hVP2 infectiousbursal disease virus (IBDV). The evolutionary history was inferred using the Neighbour-Joining method. The bootstrap consensus tree inferred from 1,000 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in <50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown next to the branches. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. The analysis involved 51 nucleotide sequences. Codon positions included were 1st + 2nd + 3rd + noncoding. All positions containing gaps and missing data were eliminated. There were a total of 336 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.[90]

type amino acid sequences in the P_{BC} loop, whereas the P_{FG} loop is more similar to the classical viruses. The P_{DE} loop differed from reference variant and classical strains with the presence of 251N and 254N, and the P_{HI} loop of genogroup 5 revealed two unique substitutions: S317K and A321P.[21] Genogroup 6 consisted of samples from Saudi Arabia with 92.26–93.64% identity to the Italian genotype that is characterized by 220H, 222Q, 253E, 254S and 321V and has 94.02–95.40% identity to Russian isolate. Genogroup 7 is composed of viruses from Australia and has two distinct groups of IBDV; the classical strains that are similar to V877 and 002-73 and antigenic variant strains that are similar to 05-5 and 08/95 viruses.[22]

Pathogenesis of IBDV

The disease strikes young chickens at 3–6 weeks of age, while sub-clinical form of infection is established in older birds. Layer type chickens are more susceptible to vvIBDV than broiler type[23] and higher mortality rates are observed in light than heavy breeds. As mentioned earlier, IBDV has two serotypes, serotype 1 and serotype 2 however serotype 1 develops IBD in chicks. Apparent

pathological lesions are observed with experimental infection on pigeon and guinea fowl.[24] The primary organ of predilection is bursa of Fabricius (BF) where majority of the B cells are in actively dividing stage in young chicks. Faeco-oral route and inhalation are the major routes of entry of the virus that replicates in gut-associate macrophages and lymphoid cells and results in primary viremia through portal circulation. Following primary viremia, the virus reaches BF by 11-hr post inoculation and after active replication in bursal follicles and B cells, the virus enters the bloodstream to cause secondary viremia. This leads to spread of the virus in other organs like kidneys and muscle tissue that leads to pathognomonic clinical signs and death. Following infection of the BF, degeneration and necrosed B-cell follicles especially IgM+ cells are detected immediately, with associated infiltration of inflammatory cells such as heterophils. As the inflammation reduces, there is necrosis and phagocytosis of heterophils and plasma cells, fibroplasia in the interfollicular connective tissue, and ultimately bursal atrophy.[25] With concurrent histological changes, molecular variations like upregulation of antiviral genes that are involved in the type I interferon (IFN) response, pro-apoptotic genes, and pro-inflammatory cytokines and chemokines, presumably from the infected B cell population appears.[26] VP2 and VP5 proteins of the virus induce apoptosis in B cells and other lymphoid cells thereby causing cell depletion.[27]

Other organs show pathological signs like splenomegaly, petechial hemorrhages on the mucosa at the juncture of the proventriculus and diffused hemorrhages in the thigh and breast muscles. Lesions in the caecal tonsils, thymus, spleen and bone marrow confirm infection with vvIBDV, with the harderian gland being severely affected following infection in the day-old chicks.[28] Repopulation of B cells in the BF happen in the recovered birds. The cells arrange in two different types of follicles either the long follicles which are the repopulated endogenous bursal stem cells in the survived birds, and can mount their own immunity or the small, poorly developed follicles lacking a distinct medulla and cortex due to the damage following infection.[29]

Immune response towards IBDV infection

IBDV causes immunosuppression in chickens, and BF is the target organ for viral replication. The stage of B cell differentiation in the BF plays an important role in viral replication as the stem cells and peripheral B cells do not support viral replication. The acute phase of the disease lasts for 7–10 days, during which the B cells are depleted in the bursal cortex and medulla, peripheral blood and thymic medulla, and the BF becomes atrophic. The viral antigen can also be detected in the peripheral lymphoid organs like caecal tonsils, spleen and thymus besides BF. The disease when not fatal leads to immunosuppression with reduced antibody response. The chickens fail to produce antibodies against other viral diseases and lead to subsequent outbreaks with the surviving birds tend to show high anti-IBDV antibody titers.

Protection against the disease does not depend solely on the humoral immunity; cell-mediated immunity through T cell involvement is also important. IgM+ B cells serve as targets for IBDV[30] and CD4+ and CD8+ T cells, along with the macrophages accumulate in the BF even as early as 1 dpi[29] with upregulation of IFN-γ, IL-8 and IL-6 transcription and apoptotic mediators like nitric oxide or TNF-α.

The role of cell-mediated immunity following IBDV infection is well established[31,32] with the localization of bursal mRNA transcription of the pro-inflammatory cytokines IL-1β, IL-6, CXCLi2 and IFN-γ together with down-regulation of transcription growth factor-β4. In vivo challenge with vvIBDV UK661 strain upregulated the transcription levels of types I, II and III IFNs as well as IL-18, IL-4 and IL-13 cytokines.[33]

Diagnosis

Clinical signs and differential diagnosis

Clinical manifestation is dependent on various factors like age, strain of the virus, maternal antibody titer, type of vaccine used, breed of the bird, etc. The incubation period is 2–3 days, after which the infected birds show distress, depression, ruffled feathers, anorexia, diarrhea and soiled vent; classical strains of virus can cause 10–50% mortality rates in infected flocks, whereas vvIBDV strains can cause 50–100% mortality. The clinical disease lasts for 3–4 days, followed by rapid recovery of the surviving birds. Few diseases, namely avian coccidiosis, Newcastle disease, chicken infectious anemia, stunting syndrome, mycotoxicosis and nephropathogenic forms of infectious bronchitis are differentially diagnosed with IBD. The BF is the principal target organ to differentiate between these diseases on postmortem examination of the dead birds. In acute form, the bursa is turgid, oedematous, and sometimes hemorrhagic and turns atrophic within 7–10 days. The bursa is atrophic in sub-clinical IBD and can be mistaken with Marek's disease or infectious anemia.

Virological diagnosis

In the acute phase of the infection, IBDV could be detected within the first 3 days post infection in the bursa. Confirmation of clinical disease or detection of subclinical form is best done by immunological assays as it is difficult to isolate the virus.

Virus isolation

During the acute phase of the disease, the bursa is collected from the susceptible chicks and a 20% bursal homogenate in PBS solution is prepared from the pooled bursa. The isolation of IBDV strains is done by infecting the embryonated chicken eggs following inoculation onto the chorio-allantoic membrane (CAM). IBDV isolation in chicken primary bursal cells from the BF has also been reported.[34] However, isolation of very virulent form of the virus is not recommended in cell culture. Death due to IBDV infection begins 3 days post-inoculation (dpi) with typical signs of hemorrhages and edema in the embryos. The best method for virus isolation is done by inoculating bursal homogenate to SPF chickens and the virus is then isolated from the bursal tissue 3 dpi. The virus is then titrated and the endpoint titers are calculated based on specific deaths by inoculating 10-day-old chicken embryos through CAM route.

Detection of viral antigens

Agar gel immunodiffusion (AGID) test detects the antigen in the bursa by placing the minced bursa from susceptible chicks in the wells of the AGID plate against known positive serum. Freeze-thaw cycles of the minced tissue release the IBDV antigens from the tissue and the freeze-thaw exudate is used to fill the wells.[35] Antigen-capture-ELISA was described for the detection of serotype 1 IBDV in which the ELISA plates were coated with mouse anti-IBDV monoclonal antibodies (Mabs) or chicken anti-IBDV polyclonal sera.[36] A panel of seven Mabs, namely 1, 3, 4, 6, 7, 8 and 9 were used in which Mabs 2 and 3 bound with classical strain but not vvIBDV strain.[37]

Molecular diagnostic tests

Sequencing the hVP2 gene together with pathogenicity testing in chickens is the most accurate and accepted method for identifying the IBDV strains. The amplification of hVP2 gene by reverse transcription PCR (RT-PCR) followed by sequencing and phylogenetic analysis represents the only valuable tool for the classification of IBDV strains.[14]

Real-time RT-PCR

Real-time RT-PCR allows IBDV differentiation based upon time and number of samples that can be tested simultaneously. The RT-PCR SYBR green technology is robust and may serve as a useful tool with high capacity for diagnostics as well as in viral pathogenesis studies. Different types of infectious IBDV strains including, virulent strain DK01, classic strain F52/70 and vaccine strain D78 were quantified and detected in infected BF and cloacal swabs by real-time RT-PCR with SYBR green dye.[38]

Loop-mediated isothermal amplification (LAMP)

LAMP compared to RT-PCR is 10 times more sensitive, rapid and specific assay and is the method of choice for field virus detection with no cross-reactivity.[39] The test is specific as 2–3 pairs of primers are used to amplify the more conserved-targeted regions.

One-step strip test

One-step strip tests based on colloidal gold labeled monoclonal antibodies were developed to detect the IBDV antigen. The test was found to be highly sensitive, specific and rapid for diagnosis of the infection in the field when compared with AGID test.[40]

Serological tests

The commonly used tests for serological diagnosis are AGID,[41] virus neutralization test (VNT),[42] or ELISA.[43] The simplest of all is AGID assay, however, its sensitivity and specificity may vary among laboratories and is time consuming. VNT has the highest specificity and at the same time, it correlates with protection. But the test is labor intensive, and moreover requires the facilities of a virology laboratory, thus impractical for routine use. For IBDV serology, an ELISA kit based on the whole virus as an alternative to AGID test involves the process of growing and purifying IBDV and again is restricted by safety considerations. ELISA kits or latex agglutination assay[44] based on the recombinant VP2/VP3 protein could be simpler and safer to produce with higher sensitivity and specificity as the recombinant proteins used in the assay is immunodominant and devoid of any nonspecific moieties as present in whole cell preparations.[7,43] As VP3 is a group-specific major immunogenic protein of IBDV, following infection with live or inactivated IBDV, the earliest appearing antibodies are directed toward VP3.[6]

Vaccination and management strategies

Strict hygiene measures, vaccination with conventional live attenuated and inactivated viral vaccines have been used to prevent IBD. However, eliminating the sturdy and the persistent IBDV particles from the farm is a difficult task as the virus remains infectious for 122 days in a chicken house and for 52 days in feed and water.[45] Therefore, routine sanitary measures must rigorously be followed to control IBDV. Disinfection may reduce the virus load and thus reduce the risk of transmission. The mechanical vectors such as mosquitoes, mealworms, and smaller rodents must be eradicated. On the farms where IBDV outbreaks have occurred, the virus may be considered endemic. Improper cleaning of the hoods will expose the young birds to the virus at an early age. Early subclinical infection is the main cause of economic loss as the disease can cause severe, long-lasting suppression of the immune system and the immunocompromised birds do not respond well to vaccination and are more susceptible to other infections. Culling the infected chickens and controlling other flocks from being infected is costly. Thus, vaccination remains the method of choice to control IBD. However, vaccine failures do occur due to the evolution of the virus even with strict vaccination practices.

An early method of prevention is exposing the young chicks to IBDV[46] and this technique although reduces mortality but often results in immunosuppression and further dissemination of the field virus. Live-attenuated vaccines based on mild field isolates after passaging in specific-pathogen-free eggs were developed. They are still widely used today in parent stock as a primary vaccine for controlling the very virulent IBD in many countries. Until the 1980s, mortality caused by IBD was effectively controlled by vaccination. With the emergence of Delaware variants in the USA in the mid-1980s and the emergence of very virulent forms of the virus in Europe and Asia in 1989 resulted in vaccination failures.[14,25,46] It is essential to prevent the infection at an early age, so that the immunosuppressive effect of IBDV could be controlled. This can be achieved by immunization of the parent stock. Inactivated vaccines along with oil-adjuvants boost the immune response and the maternal immunity may be extended to 3–5 weeks. When young chickens are to be vaccinated with attenuated vaccines, timing of vaccination is important as too early vaccination may lead to neutralization of the vaccine by MDA, and on the contrary, the birds may remain unprotective if vaccinating too late due to the low level of MDA. Monitoring the antibody level in a breeder flock or its progeny can aid in determining the right time to vaccination.[25] The MDA level can be determined by serological monitoring and the right time of vaccination could also be determined. Vaccines may be administered by intramuscular injection, by spray or by mixing in drinking water. Chickens vaccinated with IBDV in early life before 7 days of age and revaccinating with an inactivated, oil-adjuvant IBD vaccine at 18 weeks of age can produce and maintain high levels of virus-neutralizing antibody through 10 months of lay.[47] Moreover, due to early vaccination, the vaccine virus will spread in the poultry farm and indirectly could provide immune response to the other susceptible chicks.[48]

Live-attenuated vaccines are referred to as mild, intermediate, or "intermediate plus" (hot) vaccines based on the ability to cause varying degree of histopathological lesions and are suitable for mass vaccination preferably through drinking water to induce robust cellular and humoral immunity. The mild vaccines do not cause bursal damage in chicks but have poor efficacy in the presence of MDA or vvIBDV infection. Vaccines of higher pathogenicity (intermediate or "intermediate plus") may break through the high levels of maternal immunity but may produce bursal lesions, with subsequent immunosuppression leading to a secondary infection. In addition, they may not protect against infection with vvIBDV[49] or antigenic variants. Inactivated vaccines, mostly formulated as water-in-oil emulsions, are usually administered in the breeder hens for vertical transmission of high, uniform, and persistent antibody titers to the progeny.[50] Intermediate and "hot" vaccines are mostly used to overcome the MDA in young broilers. The possibility of reversion to virulence, generation of reassortment strain and vaccine reactions resulting in disease or production loss may be the few undesirable side effects. Due to these limitations, the new generation vaccines have been developed to control IBDV (Table 1).

Subunit vaccines

The major capsid protein VP2 has proved to be an important target for generating cellular and humoral immune responses against IBDV infection.[31] The VP2/3/4 polyprotein or VP2 (rVP2) alone has been expressed in different expression systems like *Escherichia coli*,[51] *Lactococcus lactis*,[52] *Saccharomyces cerevisiae*,[31,43] *Pichia pastoris*,[53] fowlpox virus,[54] baculovirus,[55] Semliki Forest virus,[56] and even plant expression systems.[57] In experimental studies, rVP2 protein can induce partial to 100% protection. The recombinant-based vaccines could allow the development

Table 1 Recent development in IBDV vaccines research

Platform	Gene targeted	Expression system	Efficacy
Subunit vaccine	Mimotope	*Escherichia coli*	100% protection against mortality[82]
	Hypervariable region of VP2	*Pichia pastoris*	100% protection upon challenge[53]
Live viral vectored	VP2/PP	Fowl pox virus	Low protection with VP2 and no protection with PP[54]
	VP2	Herpesvirus of turkey	100% protection in day-old vaccination[75]
	VP2	MDV	55% protection[7]
	VP2	NDV	90% protection[77]
SVP	VP2	*Pichia pastoris*	500 µg SVP with and without adjuvant conferred 100% protection but with moderate bursal damage[60]
	VP2	*Saccharomyces cerevisiae*	SVP alone and as a DNA prime-protein boost, 100% protection, Th1 and Th2 mixed response[31,44]
Adjuvant with IBD vaccine	Recombinant IFNs and IL-1β in combination	*Escherichia coli*	Increased humoral responses in immunologically mature but not day-old chicken[83]
	Porcine lactoferrin	*Pichia pastoris*	Increased CMI response[84]
	Chicken beta-defensin-1 genetically fused with VP2	as DNA vaccine	100% protection, Increased CD3+, CD4+ and CD8+ T-cell subtypes[64]
	Salmonella typhimurium fliC + VP2	as DNA vaccine	80% protection, increased humoral and cell mediated immunity[65]
	cHSP70 + VP2	as DNA vaccine	100% protection, increased expression of IFN-γ and IL-12, IL-10, increased ELISA Ab[31]
	VP2-4-3 and chicken IL-18	as DNA vaccine	93% protection, increased induction of IFN-γ and IL-4[85]

Abbreviations: MDV, Marek's disease virus; NDV, Newcastle disease virus; PP, polyprotein VP2-4-3; VP2, Virus protein 2; SVP, subviral particle; IFN, interferon; IL, interleukin; cHSP70, C-terminal domain of heat shock protein 70 of Mycobacterium tuberculosis; fliC, flagellin; ELISA, Enzyme linked immunosorbent assay; Ab, antibody; IBDV, infectious bursal disease virus SVP, subviral particle.

of differentiation of infected from vaccinated animals (DIVA) strategy to differentiate the vaccinated from infected ones. However, this new generation vaccine needs to be administered along with adjuvants parenterally and repeated booster immunizations increase its manufacturing cost. Till date, recombinant vaccines based on VP2 expressed in *E. coli, P. pastoris* and baculovirus have been licensed for commercial use.

Virus-like particle (VLP) based vaccine

VLPs are robust protein cages in the nanometer range that mimic the overall structure of the native virions but lack the viral genome. The trimmed VP2 and VP3 genes of IBDV generated a VLP in baculovirus expression system.[58] Attenuated pathogens are commonly excellent inducers of T cell as well as B cell responses, but as discussed earlier have chances of reversion to a more virulent phenotype. Non-infectious subunits of pathogens such as recombinant proteins, peptides or sugars are poorly immunogenic and have to be formulated with immune-stimulating adjuvants. The immunogenicity produced by a VLP-based vaccine is much better than pVP2 subunits and IBDV polyprotein expression products.[59] The VLP scaffold is produced by the electrostatic interaction between VP2 and VP3 proteins. In another strategy, 23 nm subviral particles (SVPs) in yeast (*P. pastoris*) expression system was produced that provided partial protection upon IBDV oral challenge and complete protection by intramuscular challenge.[60] IBDV SVPs expressed in *S. cerevisiae* are based on the assembly of a single protein (VP2) into 20 trimeric clusters of VP2 with T1 symmetry having a diameter of approximately 22 nm[43] (Figure 2). The SVPs-based IBD vaccine could completely protect the birds upon vvIBDV challenge and induced both humoral and cell-mediated immune responses.[31] A single shot of SVPs IBD vaccine in the hatchery could eliminate the costly, time-consuming vaccination in the field even in the presence of MDA antibodies (data not shown).

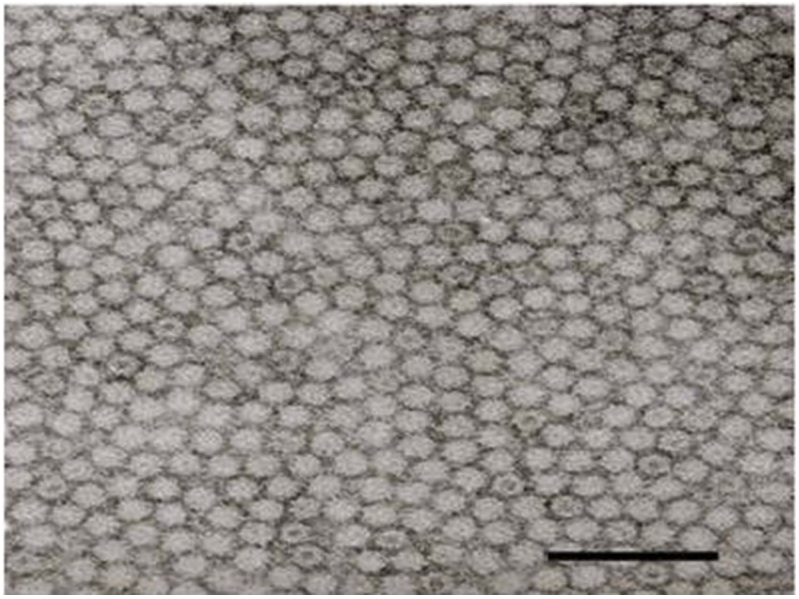

Figure 2 Transmission electron micrograph of cesium chloride gradient purified Infectious bursal disease virus (IBDV) virus protein 2 (VP2) subviral particles (SVPs), negatively stained with sodium phosphotungstate. Reprinted from.[43] Copyright 2009, with permission from Elsevier. Bar = 100 nm.
Notes: Dey S, Upadhyay C, Mohan CM, Kataria JM, Vakharia VN. Formation of subviral particles of the capsid protein VP2 of infectious bursal disease virus and its application in serological diagnosis. *J Virol Methods*. 2009;157(1):84–89

DNA vaccines

A DNA vaccine expressing VP2 can induce both humoral and cell-mediated immune responses, but the protective efficacy varies from 40% to 80% and often has resulted in bursal lesions resulting in immunosuppression. IBDV polyprotein encoding cDNA is a better candidate than VP2 encoding cDNA.[61] For better results, priming in ovo or at 1-day-old followed by boosting with inactivated vaccine or fowlpox vectored vaccine is performed.[62] In ovo delivery alone without a boost vaccine, however, did not induce sufficient protective immunity. However, the level of protection depends on the quantity of DNA used in the priming vaccine, the challenge strain of viruses used, age of the bird and route of vaccination. The failure to achieve complete protection with VP2 gene-based DNA vaccine led to improved strategies based on the incorporation of cytokines genes or cytosine-phosphate-guanine (CpG) motifs.[20] A DNA vaccine carrying VP234 gene of IBDV and IL-18 enhanced the immune response and protection efficacy to 93% against vvIBDV.[63] In a study, complete protection with a DNA vaccine encoding the IBDV VP2 protein fused with defensin (AvBD1) gene was reported with the DNA vaccine encoding VP2 protein alone providing 80% protection.[64] A chimeric DNA vaccine encoding C-terminal HSP70 of *Mycobacterium tuberculosis* fused with full-length IBDV VP2 gene induced mixed Th1 and Th2 responses[31] and completely protected the birds when boosted with SVP-based vaccine in which VP2 has been expressed in *S. cerevisiae*.[43] A TLR-5 ligand flagellin (fliC) antigen of *Salmonella typhimurium* when fused with an N-terminal of VP2 and injected as a DNA vaccine produced 80% protection but could stimulate both the arms of immunity.[65] For achieving a successful immunization with IBDV-DNA vaccine, age of bird, route of vaccine administration and virulence of challenge virus are crucial.

Immune complex vaccine

Immune complex vaccine (Icx) is a cocktail of live pathogenic IBDV strains mixed with anti-IBDV antibodies derived from hyperimmunized chickens sera or recombinant neutralizing antibody and is available commercially.[66,67] It can be administered subcutaneously to day-old chicks even in the presence of MDA, resulting in generation of active immune response without causing any vaccine-induced immunosuppression.[68,69] Icx vaccines are also used to vaccinate in ovo at day 18 of incubation using automated technology to achieve very precise vaccination. By this route of inoculation, the vaccine induces the formation of more germinal centers in the spleen, thus resulting in localization of IBDV in dendritic and bursal follicles. Post-challenge, IBDV-Icx vaccine efficacy was found to be equal to or better than that of conventional live vaccines. The principle

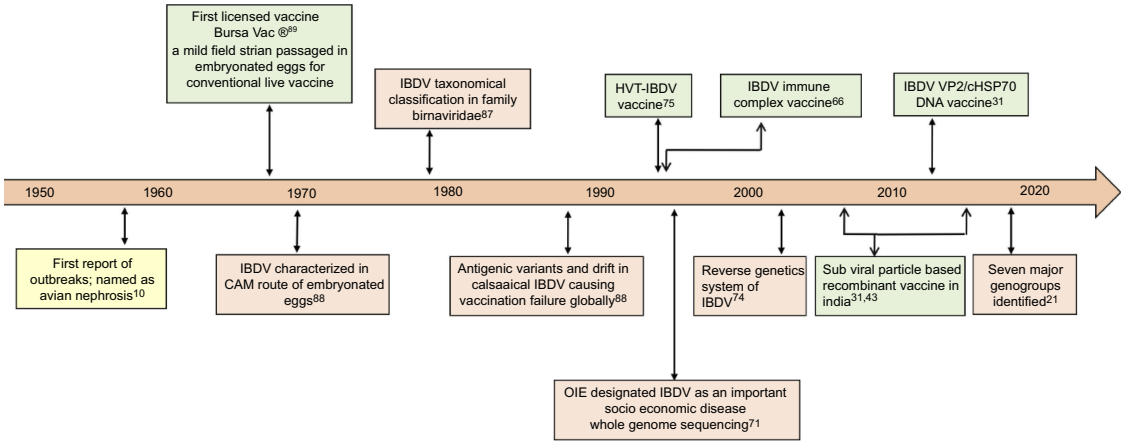

Figure 3 An overview of 60 years of infectious bursal disease (IBD) research.
Abbreviations: IBDV, Infectious bursal disease virus; HVT, Herpesvirus of Turkey; VP2, virus protein 2; cHSP70, C-terminal domain of heat shock protein 70 of Mycobacterium tuberculosis; CAM, Chorioallantoic membrane; OIE, Office International des Épizooties [World Organisation for Animal Health].

behind this technology is that IBDV antibody in Icx vaccine forms a complex with the virus and thus causes delay in virus detection (5 days) and also a remarkable B cell depletion in bursa and spleen.[70]

Reverse genetics and live viral-vectored vaccines

Understanding the genome of the viral pathogen helps in targeting the specific antigenic protein, host–pathogen interaction and also reverse engineer the virus to generate a live vaccine candidate. The whole genome sequencing of the IBDV[71] paved the way toward the development of a reverse genetic vaccine system.[72] The hypervariable region of VP2 gene was changed to an attenuated mutant IBDV from vvIBDV by site-directed mutagenesis[72,73] and then rescued by reverse genetics. The mutant virus was able to protect against both classical and antigenic variant IBDV strains.[74] There are several reports of using recombinant live viral vectors including herpesvirus of turkey,[75,76] NDV,[77] fowlpox virus,[54] Marek's disease virus[78] and avian adenovirus[79] incorporating VP2 gene with the aim of protecting the birds against IBD with a DIVA strategy. Humoral immunity plays an important role in the clearance of IBD following vaccination, with rapid infiltration of T cells into the bursa and upregulation of CMI related genes following infection with very virulent IBD.[80] The HVT-VP2 vaccine has been licensed in several countries and is found to be safe as the virus is poorly sensitive to MDA, does not produce bursal lesions and has been extensively validated in field efficacy studies.[81] This vaccine can either be used as an in ovo application or given by subcutaneous route in day-old chicks.

Conclusion

Poultry farming has grown phenomenally since the times of backyard farming to a proliferating and profitable business in many countries across the globe. Intensive poultry production system has led to the occurrence of frequent disease outbreaks with IBD figuring prominently in many countries. Although emerged 60 years back, the disease still poses an economical threat to the poultry industry worldwide. The emergence of antigenic variants and very virulent strains of the virus further complicate the field scenario that contributes to high mortality in birds. The milestone encompassing the IBD research over the years is presented in Figure 3. Recent and future developments in molecular epidemiology, development of new generation diagnosis and vaccines could greatly aid in containment of this scourge in the near future.

Disclosure

The authors report no conflicts of interest in regard to this review.

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