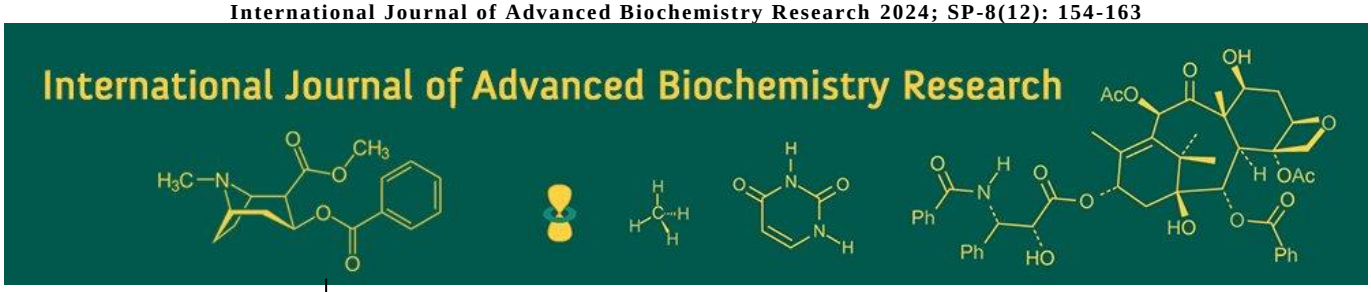

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A comprehensive overview of infectious bursal disease, a major threat to poultry health and production

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Abstract
Infectious bursal disease is an economically important and highly contagious viral disease of poultry and turkey caused by a double stranded RNA virus belonging to family birnaviridae. Among the two serotypes, serotype 1 affects young chicks between the age group of 3-6 weeks and is associated with high mortality and morbidity. The disease is characterized by prostration, dehydration, watery diarrhea, ruffled feather and hemorrhages on thigh muscles. This virus causes severe degeneration of B lymphocytes associated with severe immunosuppression thus making chicks susceptible to other secondary bacterial infestations besides unresponsive to vaccinations. Despite following different vaccination strategies, it has been difficult to control this disease because of emergence of new virus strains as a result of genetic assortment. Therefore, prevention is important and vaccination has become the principal control measure of infectious bursal disease virus infection in chickens. Conventional live attenuated and killed vaccines are the most commonly used vaccines. With the advancement technology, new generation or genetically-engineered vaccines like deoxyribonucleic acid and subunit vaccines have been used. This review has been aimed at providing the information about the history of IBD, the available diagnostic techniques, prevention and control strategies and the challenges encountered in that process.

Keywords: Infectious bursal disease, immunosuppression, live attenuated vaccines, diagnosis, control strategies

Introduction
Poultry farming has become increasingly important as a result of increased consumer demand for wholesome, economical, and high-quality poultry products that are high in protein, vitamins, and minerals [1]. Chicken is the most generally recognized poultry product in India and around the world, and it is consumed as a supplementary diet regularly by people. Poultry meat and eggs grew at a quicker rate than beef, veal, and pig meat between 1970 and 2017 [2]. Depending on farm size, poultry farming is the primary source of income for many families, and it also provides extra employment opportunities in the community. Aside from the numerous prospects in this industry, there are certain challenges, such as environmental dangers, avian flu outbreaks, outbreaks of other bacterial and viral infections, vaccination failure due to a lack of effective cold chain maintenance, and the unavailability of high- quality food. Among these barriers, one of the most important viral disease namely Infectious Bursal Disease (IBD), is wreaking havoc on poultry producers. IBD is the second most serious threat among the infectious poultry diseases [3]. Infected flocks are very contagious; morbidity is considerable, with up to 100% seroconversion following infection, and mortality is variable [4]. The disease causes substantial economic losses due to increased mortality, poor performance and immunosuppression, which increases vulnerability to other diseases and decreases response to vaccinations.

History
Infectious bursal disease (IBD) was first identified in broilers by Albert S. Cosgrove on the Delmarva Peninsula in 1957 as a clinical entity responsible for acute morbidity and death. The clinical signs observed were ruffled feathers, diarrhea, trembling and depression.

Enlarged kidneys with prominent tubular degeneration, edematous bursa of Fabricius (BF), and hemorrhagic thigh muscles were the primary lesions associated with this viral disease. This disease was termed as 'Gumboro Disease' as it was first documented in Gumboro, Delaware, USA, in 1962 [5]. Winterfeid and Hitchner recognized the viral basis of the infection in 1962 and coined the term "Nephrosis and Nephrosis Syndrome" to describe the condition [6]. The condition was termed "Nephritis and Nephrosis Syndrome" because the renal lesions were comparable to those seen in IBV (Gray strain) and Avian nephrosis. Following further examination, it was discovered that the virus that caused Gumboro disease was able to infect birds that were previously immune to Gray virus, causing alterations in the cloacal bursa [7]. By 1965, the disease had spread throughout the United States 'broiler and commercial egg production zones. The lack of specific-pathogen-free (SPF) eggs, as well as inadequacies in viral and serologic approaches, hampered early attempts to isolate the etiologic agent. By the late 1960s, the pathophysiology of Infectious bursal agent (IBA) had been clarified [8] and a virus was determined to be the etiological agent, that was later found to be resistant to a wide range of disinfectants as well as extreme pH and temperature conditions [9]. To isolate the virus in embryonated eggs and adapt it to tissue culture, reliable procedures were devised. In 1976, the agent was identified as a virus belonging to a new taxonomic category. In 1979, a Canadian scientist named Peter Dobos demonstrated the biophysical and biochemical features of IBDV and proposed the term "Bi- RNAviruses" as a new taxonomic category. The suffix bi- denotes double-strandedness and the virus genome's bisegmented structure, and "RNA" denotes the type of viral nucleic acid [10]. In 1979, it was revealed that the IBDV genome is made up of two double stranded RNA segments namely, A and B. The global distribution of the IBDV is indicated in Figure 1.

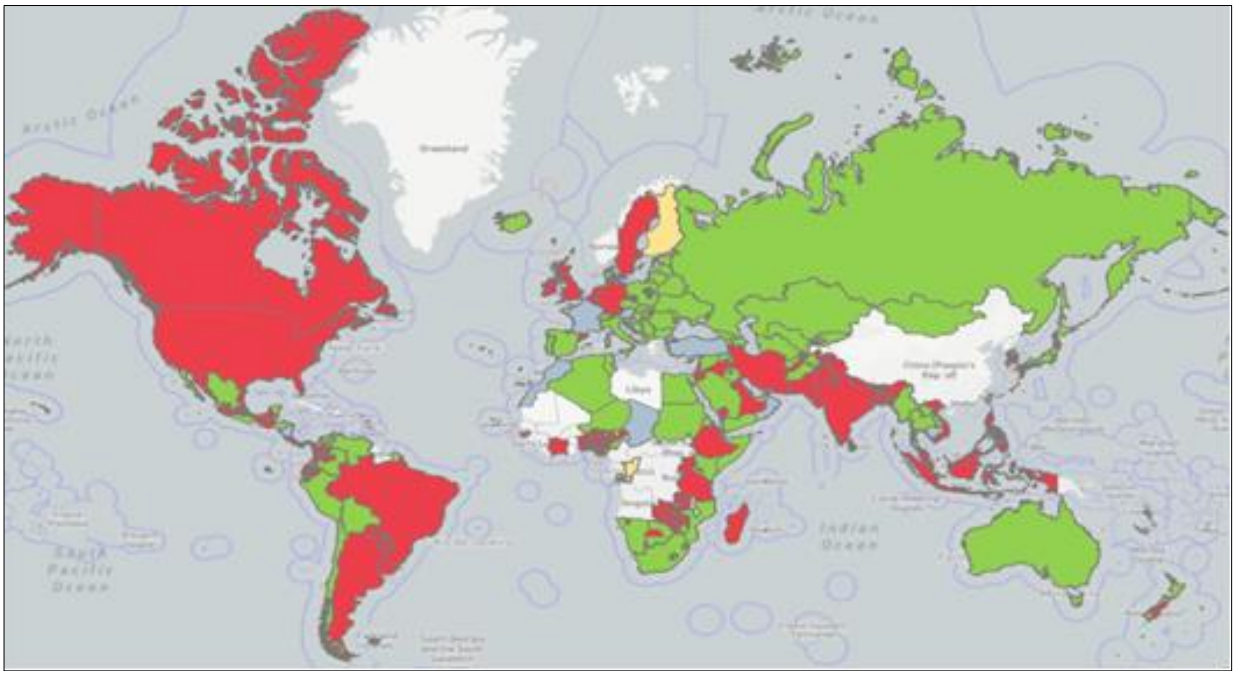

Fig 1: The global dissemination of IBD and its current status based on the most recent data spanning from (2020 to 2023), as documented in the World Animal Health Information System (WAHIS) adopted from the World Organization for Animal Health (OIE)

Etiology

Infectious bursal disease, often known as 'Gumboro disease' first discovered in Gumboro, Delaware United States of America by Cosgrove [5] is an acute, highly contagious and immunosuppressive viral disease of young chickens and turkeys caused by a non-enveloped double-stranded RNA virus from the Birnaviridae family [11]. IBDV has two linear, double-stranded RNA segments. It replicates in the cytoplasm of the host cell and is aided by a virion-associated RNA polymerase. The Birnaviridae family includes the genera that infect chickens, fishes, and insects. The IBDV infections mostly affect the cloacal bursa, although it can also replicate in other lymphoid organs such as the spleen, thymus, Harderian gland, and caecal tonsils. The virus targets actively multiplying and differentiating B-cells, resulting in age-dependent immunosuppression [5]. B-cell immunosuppression is severe and permanent in chicks infected before they reach the age of one week. The immunosuppression so caused by IBDV infections not only increase the chicken's susceptibility to secondary opportunistic infections like gangrenous dermatitis, inclusion body hepatitis, chicken anemia agent, respiratory diseases, and E. coli infections, but also frequently meddle with effective immune responses to vaccination.

Genomic Structure

IBDV is a non-enveloped, icosahedral, double-stranded RNA virus with a bi- segmented genome that belongs to the genus Avibirnavirus and family Birnaviridae. IBDV has a bipartite dsRNA genome (segments A and B), which is packed into a single-virus particle with a diameter of around 70 nm [12]. Segment-A is made up of 3261 nucleotides with two open reading frames (ORFs), the smaller of which encodes a non-structural protein known as VP5 and the larger of which encodes a polyprotein that is processed co-translationally by the viral protease VP4 to generate the protein capsid precursor pVP2, VP3, and serine protease VP4 [13]. The VP2 and VP3 form the virion's primary structural proteins, whereasVP4isavirus-encodedprotease. Serial cleavages at the carboxy terminus of pVP2 produce

mature VP2 and four peptides that stay attached to the virion. Segment-B is monocistronic, encoding only VP1, an RNA-dependent RNA-polymerase (RdRp) [14] Figure 2.

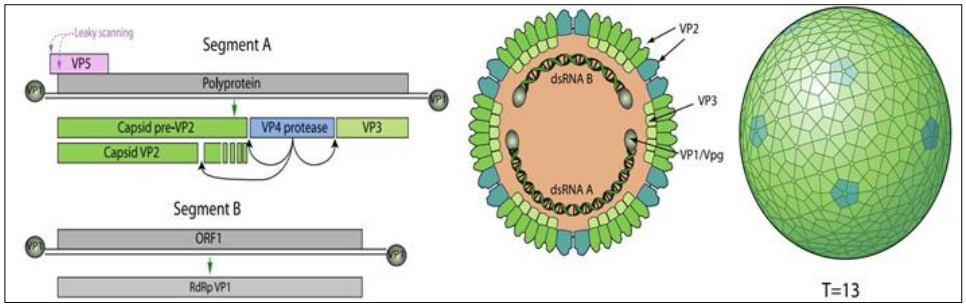

Fig 2: Morphology of Infectious bursal disease (IBD) Virus Source: Viral zone

Serotypes

IBDV has been categorized into two serotypes (1 and 2) based on virus neutralization assay. Within each serotype, both exhibit substantial antigenic variation [15]. Serotype-1 is pathogenic in chickens and is subdivided into two types based on antigenicity (classical and variant) and pathogenicity (attenuated, virulent vIBDV, and extremely virulent vvIBDV). Within serotype-1 of IBDV strains, however, death patterns vary, with no mortality for variant strains, around 20% mortality for classical strains and over 50% mortality for vvIBDV [16]. In chickens and turkeys, serotype-2 viruses are inherently avirulent and do not produce any clinical illness. Serological evidence ofserotype-1 of IBDV infection in wild birds, on the other hand, implies that wild birds may play a role in IBDV epidemiology by acting as viral reservoirs. Serotype-2 of IBDV has been reported as most prevalent among variety of free-living wild bird species, with turkeys being the natural host [17].

Transmission

The Gumboro virus being a hardy virus is natural inhabitant of the poultry house, which is the first crucial thing to comprehend. Accordingly, the chicken's Bursa of Fabricius becomes infected once the levels of maternal derived antibodies (MDA) drop below threshold limit, and the infection can spread from one chicken to another or from one flock to another. This increases the risk of the co-housed chickens contracting a virus from the poultry house or from a different animal [18]. There are several factors that explain the type of an IBD transmission, but four of them are particularly important in the context of farms:

1 Farm IBDV
2 Virus pressure
3 Passive Immunity (MDA)
4 Active Immunity

Farm IBDV

The Gumboro virus, or Farm IBDV, is already prevalent when day-old chicks are introduced and is very likely to infect them when they become susceptible. Because of the Gumboro Disease virus's great resistance, it is quite likely to stay in the environment even after rigorous cleaning and disinfecting measures, meaning that it will already be present within the house when the day-old chicks are let loose on the floor. Numerous factors, notably the previous flocks in that house, will affect how the IBDV "population" builds up. Thus, if the preceding flocks were not adequately protected or experienced an epidemic, the field virus will persist for the following flock.

Virus Pressure

The amount of virus posing a challenge to the poultry is indicated by the virus pressure, also known as challenge pressure. The management system used at the farm (all-in, all-out, accumulated litter, multiage, cages, etc.), the standard of cleanliness and disinfection, and the farm's location in relation to other nearby farms are all related to virus pressure. Virus pressure varies throughout the growth phase as well. If IBDV is able to infect some chickens, then these chickens will spread the virus through shedding and viral replication, which will raise the pressure of the virus. The danger of infection, clinical effects, and economic burden are all exacerbated by a high viral pressure.

Passive Immunity

Passive immunity supplied by Maternally Derived Antibodies (or MDA) serve a critical function in containing virus pressure and preventing infection if it is present in the chicken in a sufficient concentration at a sufficient level. The MDA level increases during the first few days after hatching because immunoglobulins that are still in the yolk sac are released into the bloodstream, then decreases over time and with the rate of chicken growth until it reaches a non-protective level that increases the chickens' susceptibility with age.

Active Immunity

Active immunity induced by administration of a vaccine will develop according to the vaccine(s) employed, the quality of application and immune status of the chickens at the time the vaccination. All live Gumboro vaccines, whether theyare attenuated, immune complex, or recombinant vector types, are used to promote immunity against Gumboro Disease. Consequently, they all need to replicate to 'work'. The replication of a live Gumboro vaccine depends on the right timing of administration because of interference with MDA. The vaccine virus will be neutralized if provided too soon when the levels of MDA are high, but if administered too late, there is a wide opportunity for farm IBD virus to infect and cause disease to the flock. This problem of timing has been solved by the development of Gumboro hatchery vaccines (Immune Complex) that have the capacity to overcome interference from passive immunity whatever the level (Figure 3).

Pathogenesis

The disease mainly affects young chickens at 3–6 weeks of age, while older birds get infection in sub-clinical form. Broiler type birds are less susceptible to vvIBDV infection than layer type chickens. Following infection, IBDV is carried to the BF by infected macrophages, where the virus engages in intracytoplasmic replication in IgM+ B cells [19]. The stimulation of macrophages increases interferon (IFN) production in the BF. This is accompanied by the release of proinflammatory cytokines such as interleukin-6 (IL-6) and nitric oxide (NO) [20]. The bursal lesions may then worsen because of these cytokines. Furthermore, it has been reported that both infected and nearby healthy B-cells may undergo apoptosis because of the IFN-produced during IBDV infection [21]. At days 7–21 pi, there is a significant reduction in the IgM+ B-cell population relative to IgA and IgG B cells, as determined by flow cytometric analysis. However, the mesenchymal derived reticular cells residing in the bursal cortex, and periarteriolar lymphoid sheaths, germinal Centre (GC) and red pulp of the spleen have been reported to be relatively resistant to IBDV. The disappearance of bursal follicular dendritic cells in IBDV infection is thought to result from the absence of B-cell proliferative environment [22]. The caecal tonsils and bone marrow may aid in the replication of IBDV at a later stage. Inflammation of the BF starts to manifest 48 hours after infection (pi), and cytolytic alterations start to become noticeable in all infected bursal IgM+ B cells on days three to four post infection [23].

Immune Responses to IBDV

1. Innate Immune Response

IBDV, like many other infectious diseases, triggers an early innate immune response that is followed by an adaptive immunological response. Virus-activated macrophages are the source of innate immune responses. Macrophages are the first cells that encounter IBDV. In fact, it's thought that gut-associated macrophages play a role in IBDV transportation from the digestive tract to the BF and other lymphoid tissues [24]]. Macrophages and other immune cells, such as $CD4^+$ and $CD8^{+,}$ penetrate the site of viral replication i.e. BF, during the acute phase of IBDV infection. Gene transcription of pro-inflammatory cytokines (IL- 1β, TNF-α, IL-6, and IL-8) in the BF has been correlated with IBDV-activated intra-bursal T-cells. Systemic levels of IL-6, as well as other pro-inflammatory cytokines and chemokines, were noted during the acute phase of an infection [25] resulting in a "cytokine storm" presumably causing immunopathology and high mortality rates. Intra- bursal T-cells generate IFN-γ which is a powerful activator of IL-12, which causes splenic macrophages to release more pro-inflammatory cytokines and inducible nitrogen oxide (iNO) [26]. Moreover, IBDV-infected macrophages and bursal macrophages that are directly exposed to viral proteins release significant amounts of pro-inflammatory mediators, which induce severe bursal tissue destruction. Activation of immune cells and production of cytokines differ depending on the strain of the IBDV involved in the pathogenesis. When vvIBDV infects bursal macrophages, they display an increased level of activation compared to classical (cIBDV) or variant (vIBDV) IBDV infection [20]. In chickens infected with the intermediate strain of IBDV, bursal macrophages have been shown to increase the production of mRNA encoding the cytokines IL-1, IL- 6, IL-18, and iNOS. When compared to vIBDV strains, cIBDV strains expressed higher levels of innate (IFN-α and IFN-β) pro-inflammatory cytokines and immunological mediators (IL-6 and iNOS). The expression of chemokine genes, like IL-8 and MIP-α, was also higher in birds infected with classical strains.

Adaptive Immune Response

IBDV stimulates significant adaptive immune responses against itself while suppressing immunological responses to other infections. Despite the fact that IBDV only infects immature B-cells in the BF, considerable clonal proliferation of IBDV mature B-cells has been observed after infection with the virus [27]. In addition, IBDV promotes cell-mediated immunity, which is critical for virus clearance and recovery. The presence of IL-2 and IFN-γ in bursal tissue strongly suggests that T-cells have been activated, emphasizing the importance of IBDV-specific cytotoxic T cells in virus clearance [28].

Immuno-suppressive Cytokine Modulation by IBDV

A lytic infection of B-cells by IBDV causes the destruction of antibody-producing cells in birds, leading to immune suppression. In addition, it was found that splenic macrophages may act in a protective manner by reducing mitogen-induced lymphoproliferation of chicken splenocytes infected with IBDV [29]. Several inhibitory cytokines have been identified as being produced by monocytes and macrophages, including IL-10 and TGF-β in mice. The cytokines IL-4, IL-10, IL-13, and TGF-β limit the involvement of macrophages in the inflammatory process by decreasing the production of pro-inflammatory cytokines including IL-1, TNFα, IL-8, and IL-6 [30]. Despite the virus causing lytic infections in B-cells, the destruction of antibody-generating cells is considered one of the major reasons for immunosuppression caused by IBDV. The importance of macrophages and suppressor T-cells in immunosuppression has also been recognized. The suppressor T-cells retrieved from BF's have been shown to strongly inhibit the proliferation of normal spleen cells *in vitro*, while other splenic T-cells do not limit this mitogenic response [31].

Diagnosis

Clinical Signs and Differential Diagnosis

Clinical manifestation is influenced by several variables, including age, virus strain, maternal antibody titer, vaccine type, breed of bird, etc. After the incubation period of 2-3 days infected birds display signs of distress, depression, ruffled feathers, anorexia, diarrhea, hemorrhages on thigh muscle and a soiled vent (Figure. 4). Classical virus strains can cause mortality rates of 10–50% in infected flocks, whereas with vvIBDV strains can mortality can go as high as 50– 100%. IBD is variably diagnosed with few diseases, including avian-coccidiosis, Newcastle disease, chicken infectious anemia, mycotoxicosis, and nephron-pathogenic types of infectious bronchitis. Postmortem examination of the BF is the main target organ for differentiating IBD from these diseases. The bursa is turgid, oedematous, and occasionally hemorrhagic in acute form, and it goes atrophic in 7–10 days. In sub- clinical IBD, the bursa is atrophic and might be misinterpreted for Marek's disease or infectious anemia. During the acute phase of infection, IBD may be found within the first three days in the bursa following

infection. Since it is challenging to isolate the virus, immunological testing is the best way to confirm a clinical condition or discover a subclinical form.

Virus Isolation

Bursa of susceptible birds is homogenized in a 50 % w/v suspension in phosphate buffered saline (PBS). The homogenate is centrifuged and the supernatant harvested is inoculated in 9 to 10 day old embryonated chicken via chorio-allantoic membrane (CAM) and is the most sensitive diagnostic method for virus isolation. Death within 3 days post inoculation has been observed with classical signs of hemorrhage and edema in the embryos [32].

Serological Identification

Several serological test like AGID, ELISA and VNT are used to detect the antibodies generated after infection with IBDV. These tests are also used to monitor the vaccine response and for diagnosis of infection in un-vaccinated flocks. ELISA is the most used test for detection and quantification of IBDV generated antibodies to check natural field exposure and decay of maternal antibody titer. It is simple, economical and can be used on many samples at the same time. The VNT titers accurately corelate with the antibody titers that protect the chickens against IBDV. VNT is carried out in cell cultures. Compared to the AGID test, this is laborious and expensive, but detects antibodies more accurately. It has the ability to distinguish between IBDV serotypes 1 and 2 or to evaluate vaccine reactions with this sensitivity [33].

Viral Antigen Detection

Viral antigens can be demonstrated by the agar-gel precipitin assay or by the antigen capture enzyme-linked immunosorbent assay (AC-ELISA). AGID is the one of tests recommended by the OIE for the trade at international level. The bursal tissue sample is minced and taken in the wells of AGID plate against known positive serum. Minced bursal samples should be Freeze-thaw to improve the release of IBDV virus from the infected bursal tissue. When a distinct precipitin line, or "line of identity," forms between the tested serum and the positive control antiserum and the antigen in the center well, the results are considered positive; however, if no line forms, the results are considered negative. If no clear precipitin line forms at the positive control well or if a suspicious reaction is seen at the tested serum well, the test is repeated [34].

AGID

The AGID test can also be used to measure antibody levels by using dilutions of serum in the test wells and taking the titre as the highest dilution to produce a precipitin line [32]. This can be useful for measuring maternal or vaccinal antibodies and for deciding on the best time for vaccination; however, this AGID quantitative determination has now been largely replaced by the ELISA.

ELISA

The basic idea behind ELISA is that after adding the substrate, an enzyme is coupled to an antibody, which then binds to a particular antigen. If there are antibodies present, they will attach to the antigen after a serum sample is added. The antibody will bind to the substrate and respond if the sample is positive. As a result, the positive samples will turn color. The ELISA is the most rapid and sensitive method and presents the fewest variations due to the viral strain used as an antigen. It is economical, easy, and quick and evaluates numerous samples simultaneously. However, is unable to distinguish between the antibodies unique to the two serotypes [7].

Molecular Diagnostics

Reverse-Transcription Polymerase Chain Reaction (RTPCR) is one of the most important and commonly used molecular method to detect the genome of IBDV. Reverse-Transcription Polymerase Chain is employed to detect viral RNA in homogenates of infected organs or embryos Without considering the vitality of the virus present. As no growth of virus is needed before amplification, it detects the genome of even those viruses that do not grow in cell culture. Reverse- Transcription Polymerase chain reaction involves three steps, Extraction of nucleic acid (RNA) from sample tissue, conversion of RNA to cDNA by reverse transcriptase and amplification of cDNA by PCR [33].

Extraction of RNA

IBDV RNA is isolated mostly by two methods, proteinase K digestion-based approach and acid-guanidium-phenol-chloroform. In former approach 700 µl of bursal homogenate is added with 1% sodium dodecyl sulphate and 1gm/ml of proteinase K and incubated for 60 minutes at 37 °C. Nucleic acids are harvested from the final aqueous phase by ethanol precipitation and are resuspended in RNase free distilled water or a suitable buffer. Water-diluted RNA should be kept frozen at a temperature below -20 °C until use.

1. **First Strand cDNA Synthesis**

The extracted RNA is used to synthesis first strand of complementary DNA in thermal cycler by using reverse transcriptase enzyme.

2. **Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)**

RT-PCR works on the VP2 gene, as it is major structural protein which determines the antigenicity of the virus and is responsible for the production of neutralizing antibodies in the chicken. VP2 contains the hypervariable region that displays greater amount of variation in amino acid sequence between strains. For amplification of 743 bp fragment of VP2 hypervariable region, two primers specific to that particular region are used. The amplified product is detected by using gel electrophoresis [34].

Prevention and Control

Despite disinfection, the infectious bursal disease virus persists on poultry farms due to its high contagiousness and resistance to inactivation [7]. As a result, vaccination is especially crucial to limit the prevalence and effect of IBD in the poultry industry, even with stringent biosecurity procedures (e.g., ‘down time' between broods, all-in/all-out production, cleaning and disinfection of premises and equipment). Breeder flocks have traditionally been hyper-immunized with live and killed vaccines to pass on high maternal antibody titers to their offspring [35].

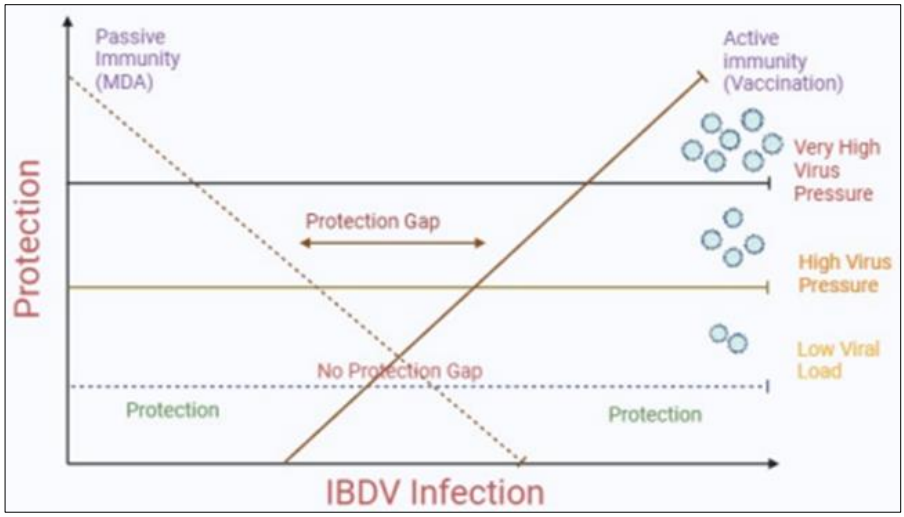

Fig 3: Schematic representation of immunity gap between waning of Passive immunity and onset of Active Immunity.

Fig 4: (A) Bursal hemorrhages and Spleenomegaly. (B) Hemorrhages on Thigh muscles

Chicks are protected from early immunosuppressive infections for 1 to 3 weeks by passive immunity; however, protection can be extended to 4 or 5 weeks by increasing immunity in breeders using oil-adjuvanted vaccines. Maternal antibody levels may vary significantly in a flock due to differences in breeder vaccination programs, the age of the breeder flocks supplying progeny, and normal variations between hen's titer levels in the same flock. As a result, the timing of broiler immunization in relation to diminishing maternal antibodies is crucial in order to avoid residual maternal antibodies neutralizing the vaccine virus [36]. To establish the best timing to vaccinate a breeding flock or its progeny, serological monitoring of antibody levels is frequently required.

Despite the existence of maternal antibodies to IBDV, field isolates classified as "variant" strains are capable of causing bursal lesions in vaccinated birds [37]. With the subsequent manufacture of IBDV variant-derived vaccines, common epitopes between IBDV pathotypes were reported. Classic strain vaccines provided only little protection against variant IBDVs, whereas variant strain vaccines provided protection against classic strains as well as homologous and heterologous variation strains [38]. In order to extend the spectrum of antigenic subtypes and elicit a stronger immune response, commercially available live and killed vaccines have been reformulated to contain both classic and variant virus strains [39]. However, vaccine efficacy on the other hand, is greatly dependent on the vaccine dose and strains, as well as the route of administration, the optimal vaccination period, and maternal antibody levels [38]. New IBDV strains have been reported that can bypass maternally derived and active immunity generated by commercial IBDV vaccinations. Classical live attenuated vaccines may provide wide, lifelong protection, but they also contain residual pathogenicity and have the potential to return to virulence. There are variety of commercially available live-attenuated virus vaccines that differ in virulence and antigenicity. Vaccine strains are classified as mild, mild intermediate, intermediate, intermediate plusor "hot" in terms of virulence, depending on their level of attenuation. Mild type vaccines are ineffective against vvIBDV and are easily neutralized by MAb. These are primarily used for breeder vaccinations, but because of their high sensitivity to homologous maternal antibody interference, they are usually given between four and eight weeks of age, when maternal antibodies have begun to fade. Antigenicity and efficacy against MAb and vvIBDV are better with intermediate, intermediate plus, and hot vaccines as they can breakthrough in the residual MAb, which is not the case with mild type vaccines [40]. There is, however, a danger of the less attenuated hot vaccines reverting to a virulent strain, as well as additional adverse effects such as vaccine-induced bursal injury or immunosuppression [24].

IBDV has been difficult to control despite vigorous vaccination regimens for the following reasons:

1. Induction of varying levels of immunosuppression by live-attenuated IBD vaccines increasing the vulnerability of birds to secondary infections.
2. Residual pathogenicity associated with live-attenuated IBDV vaccines.
3. Limited cross-protection offered by these live attenuated IBDV vaccines.
4. Because of less homology inVP2 based genes between the virus strains

5. Enhanced genetic diversity because of antigenic variation between the virus strains.

Potential of live attenuated vaccines to revert to virulence resulting in the generation of new serotypes.

The safety and cross-protective efficiency of these vaccines remain a cause of concern, thus warranting evaluation of the safety and protective efficacy of various live-attenuated IBD vaccines being used in a specific geographical region. Different vaccine strategies which are followed nowadays are depicted in Table 1.

Table 1: Recent advancements in the field of vaccines for Infectious Bursal Disease virus.

Vaccine Type	Gene targeted	Expression system	Efficacy
Live Viral Vectored	VP2 VP2 VP2	Fowl pox virus Herpesvirus of turkey NDV	Low protection [41] 100% protection in day old vaccination 90% protection [42]
SVP	VP2 VP2	Pichia pastoris Saccharomyces cerevisiae	100% protection with little bursal damage 100% protection with mixed response
Subunit Vaccines	Hypervariable region of VP2 Mimotope	Escherichia coli Pichia pastoris	100% protection upon challenge [43] 100% protection against mortality [44]
Adjuvant with IBD vaccines	Chicken beta-defensin-1 genetically fused with VP2 cHSP70 + VP2 VP2 and VP3 and Cholera toxin B adjuvant	As DNA vaccine As DNA vaccine As DNA vaccine	100% protection, Increased CD3+, CD4+ and CD8+T-cell subtype 100% protection, increased expression of IFN-γ and IL-12, IL-10 [45] Both cell mediated and Humoral response [46]

Subunit Vaccines

The key protective IBDV antigen, viral structural protein VP2 (rVP2), whose neutralizing epitopes depend on conformation, has been the subject of reports throughout the past few decades. Chicks exposed to denatured VP2 do not develop protective immunity, while chicks exposed to denatured and renatured VP2 do not also develop neutralizing antibodies [47]. Escherichia coli, yeast, fowl poxvirus baculovirus, Semliki Forest virus, and even plant expression systems have all been employed as different expression platforms. In order to effectively purify proteins, either the polyprotein gene or the VP2 encoding region alone have been employed for expression. IBDV empty capsids were found to provide better protection than the tubular structures that developed after expression in a baculovirus-based system. A fusion protein consisting of VP2 and chicken interleukin-2 has been developed as a potential vaccine and is considered to enhance immunogenicity [48]. The multi-mimotope protein r5EPIS, which was created to contain monoclonal antibody binding peptides specific to IBDV, promised to be a novel subunit vaccination candidate for IBDV in a mimotope vaccine method. Three VP2 subunit-based vaccinations have been released into the market in different nations to date. These vaccines either use the baculovirus system, E. coli, or the yeast Pichia pastoris to express VP2. Similar to inactivated vaccines, the use of such vaccines is constrained by the need for parenteral delivery and booster shots. The development of a DIVA technique to distinguish vaccinated flocks from infected ones may be made possible by recombinant subunit vaccines based only on the production ofVP2, as VP3 of the IBDV is also a potent antigen and may induce anti-VP3 antibodies that are only found in infected flocks [49].

DNA Vaccines

Immune response to a foreign antigen is induced by transfer of naked DNA, encoding the target gene, into host cells. This approach overcomes the problems caused by the organism's pre-existing specific antibodies to the antigen by promoting the formation of specific antibodies as well as cytotoxic T cells following intracellular expression of the target antigen. Several studies were conducted on the development of a DNA vaccine, with varying degree of success, to effectively trigger an immunological response in chickens. IBDV polyprotein encoding cDNA appeared to perform better than VP2 and co-administration of IBDV-specific cDNA and interleukin-2 or interleukin-6 encoding DNA increased vaccine efficacy [50]. It has been suggested that DNA vaccines might be used to prime embryos or infants as young as one day old. This could then be followed by an inactivated vaccine, or a vectored vaccination boost to produce immunity against a virulent challenge infection. It was found that in ovo delivery alone was insufficient to establish protective response. The oral administration of the IBDV cDNA vaccine has been attempted with varying degrees of effectiveness using bacteria such as Lactococcus lactis, Salmonella typhimurium, and E. coli, but the obstacles to the expressed viral protein's secretion or translocation across the bacterial cell wall may be the limiting factor [51].

Live Viral Vector Vaccines

Vector vaccines are genetically modified vaccinations in which a gene from one organism—the donor—is placed into the genome of another organism—the vector—to trigger a protective immune response against both organisms. Among others, Newcastle disease virus, fowl poxvirus, herpesvirus of turkey (HVT), Marek's disease virus, avian adenovirus and T4 bacteriophage have been used as vector viruses for expressing VP2, the only antigen inducing protective immunity to IBDV. HVT is being used since decades as a safe and effective vaccine against Marek's disease; as it is not vulnerable to interference with maternally derived antibodies, HVT has been proposed as a vector for IBD and other diseases. In the meantime, several "HVT plus IBDV-VP2" vector vaccines have been developed -in-ovo or subcutaneous administration to 1-day-old chickens [52]. In the study described by Le Gros*et al* maternal immunity, which prevents the live IBDV vaccine from replicating in a control group, had no discernible impact on the effectiveness of the vector vaccine. At the age of 30 days, 93% of the population was protected from a challenge with

vvIBDV. Remarkably, it was also noted that this vector vaccine offered protection against a challenge with mutant IBDV. Vector vaccines that just express VP2, like recombinant subunit vaccines, could facilitate the creation of a DIVA method.

Immune Complex Vaccines

Immune complex (ICX) vaccines are formulated by coupling the target antigen with antibodies against that antigen. This complex enhances the immune response by prolonging the exposure of the antigen to the immune system by delaying viral replication due to the presence of specific antibodies in the icx formulation and considerably by longer vaccine viral persistence which contribute to limit the colonization of bursa tissues by ibd viral strains. The immune complex vaccines work through following methods.

1. **Antigen selection**: Specific proteins from the IBD virus that induce protective immunity are identified. These antigens are typically proteins present on the surface of the virus or involved in its replication cycle.
2. **Antibody production**: Antibodies against these antigens are generated. These antibodies are derived from hyperimmune sera obtained from previously vaccinated or infected birds.
3. **Complex formation**: The antibodies are then complexed with the antigens. This can be achieved through chemical conjugation or genetic fusion techniques, ensuring that each antigen molecule is bound to multiple antibody molecules.
4. **Immune response**: Upon administration, the immune complex is recognized by antigen presenting cells (APCs) such as dendritic cells. The antigen is internalized by the APCs and processed and presented to T cells and initiating an adaptive immune response.
5. **Immune activation**: The presence of the antibody antigen complex enhances the activation of B cells and the production of antibodies specific to the IBD virus. This leads to the generation of a robust humoral immune response including the production of neutralizing antibodies that can prevent viral infection.
6. **Memory response**: The immune system retains a memory of the IBD virus antigens, allowing for a rapid and effective immune response upon subsequent exposure to the virus.

Immune complex vaccines have been shown to elicit strong and long-lasting immunity against IBD. They offer advantages such as improved immunogenicity and prolonged antigen exposure and enhanced targeting to immune cells. The icx vaccines overcome the problem of variable level of maternal derived antibodies to IBDV which interfere with conventional live vaccines, hence showcasing its potential for improved animal health and productivity in the poultry industry [53].

Multiepitope Vaccines

Multiepitope vaccines against Infectious Bursal Disease (IBD) represent a cutting-edge approach in immunization strategies. These vaccines are designed to target multiple epitopes, which are specific parts of the virus that are recognized by the immune system. Both major and minor capsid proteins (VP2 and VP3) were used to construct the vaccine along with CD8+, CD4+ T-cell, B-cell epitopes and a Cholera Toxin B adjuvant using a reverse immunoformatics approach connected, using appropriate flexible peptide linker to elicit a broader and more robust immune response compared to traditional vaccines. These vaccines were evaluated for antigenicity and immunogenicity and are believed to be quite efficient. It is also believed that these vaccines elicit a significant antibody titer and potentially offer better protection against various strains of the IBD virus, enhancing the effectiveness of vaccination programs in poultry. The development of multiepitope vaccines is a valuable avenue in the ongoing global endeavor to combat IBD and improve poultry health worldwide [46].

Conclusion

Although identified more than 50 years ago, IBDV continues to pose a serious threat to commercial poultry all over the world. Despite strict bio-security measures and use of conventional inactivated and live IBDV vaccines, IBD has been difficult to control due to emergence of new antigenic variant and very virulent IBDV strains in the late 1980s. Furthermore, the major problem in early vaccination against IBD with live vaccines is the interference of maternally derived antibodies with vaccine uptake. Recent advancements in vaccination technology such as in *ovo*-vaccines and live viral vector vaccines have improved the effectiveness of the IBD vaccines even in presence of high titers of maternally derived antibodies [47]. Nowadays more and more countries are adopting in ovo vaccination method as a choice of immunization against major poultry diseases like Merk's disease and IBD. An effective and long-lasting IBD prevention and control regimen may soon be made possible by the vectored vaccines. However, it has been speculated that in practice it may be challenging to maintain high efficacy "because these strictly regulated recombinant vaccines cannot easily adapt to meet the emergence of very virulent strains of both IBDV and MDV, apparently induced by the extensive numbers of vaccinations performed against these diseases [48]."This assertion could be contested in the context of IBD given the lack of epidemiological understanding of the emergence of new IBDV strains with increased virulence and the possibility that the VP2 gene insert in some genetically modified vaccines could be swapped out rather than attenuating the newly emerging virus. However, persistent attention must be paid to field viruses and the disease's natural course.

Ethics approval and consent to participate

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Consent for Publication

Not applicable.

Availability of data and material

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Authors' Contributions

Jan Mohd Muneeb: Conceptualization and writing, Neeraj K Pawaskar: Writing and supervision, Nadeem Shabir, Muteeb-ullah-Rafiqui & Rukkiya Siddiqui: Review and editing.

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