Control and prevention of infectious bursal disease: a review

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Infectious Bursal Disease (IBD) is a highly contagious, globally occurring acute viral poultry disease caused by a bisegmented, double stranded RNA virus that belongs to the genus *Avibirinavirus* family *Birnaviridae*. The disease is economically significant to the commercial poultry industry through the mortality, reduced weight gain and condemnation carcass due to marked haemorrhage in the skeletal muscle as well as immunosuppression. The re-emergence of IBD in variant or highly virulent form in different parts of the world during the last couple of decades, have demanded further research efforts in understanding the added complexity of the disease process and the means to control it. Control of the disease has been through exclusion or eradication of chickens via all-in/all-out procedure and genetic selection of chickens resistant to the disease. At present, the disease is controlled by the combined use of live virus and inactivated oil emulsion vaccines. But these vaccines are not always safe as they may not contain the required immunogens present in the variant strains prevailing in that area. Thus, new technologies and second-generation vaccines including rationally designed recombinant and subunit vaccines have been developed. Supplementation of IBD vaccinated chickens through feeding *Azadirachta indica* (Neem) dry leaves powder and also supplementation of ascorbic acid were believed to enhance the immune response of infected chickens. The use of anti-viral drugs such as recombinant interferon alpha, Ribozyme R4 has been tried by researchers but the result is still guarded and needs further investigation. While the emphasis is on prevention rather than cure, there is not much one can do with the infected flocks once IBD outbreak had occurred in the farm. In addition, eliminating the sturdy and persistence IBD virus (IBDV) particles from the farm is by no means an easy task. In practice, there is little value in treating the IBDV-infected birds because of the incurred cost. Culling the infected chickens and prevent other flocks from being infected is a sensible approach, though costly. In other words, now, vaccination remains as the principal method to curb the disease. However, even with stringent vaccination practices, the farm is still not free from the threat of IBD because vaccination against an IBDV strain may not fully protect the birds from being infected by other strains. New technologies and next-generation vaccines such as Recombinant and DNA-based vaccines do have added advantages over conventional vaccines but their relevance and level of protections is not yet validated at commercial poultry farm level. Therefore, the current status of IBD control and prevention effort is highlighted and discussed.

Keywords: Avibirinavirus; IBDV; Immunosuppression; Vaccines

1. Introduction

Infectious Bursal Disease (IBD) is a highly contagious, globally occurring viral poultry disease. The disease was first reported by Cosgrove, who in 1962 observed a disease, affecting chickens on farms in the neighbourhood of Gumboro, Delaware, USA (1). Thus, Gumboro disease became synonymous for the condition. The virus causing IBD suppresses the immune system of affected birds by damaging organs of primarily the humoral cell defence, particularly bursa of Fabricus (BF), hence alternatively named (2).

Infectious bursal disease has worldwide distribution, and the effects of the disease are economically significant to the commercial poultry industry (3; 4; 5) through the mortality, reduced weight gain and condemnation carcass due to marked haemorrhage in the skeletal muscle (6; 7). The domesticated hen (*Gallus gallus*) is the only species for which IBD virus (IBDV) has been reported to induce clinical disease. However, some reports in serological surveys in wild birds (8) suggest their role as a reservoir. Currently, IBD becomes a problem in the poultry industry worldwide (9). Until 1987, the field strains of IBDV were of low virulence and caused only 1% to 2% mortality (9). However, new IBDV strains emerged and able to cause up to 5% specific mortality in USA (10). Meanwhile, IBD outbreaks that caused high mortality of 50% to 60% in the laying hens and 25% to 30% in the broilers were reported in Europe and Japan, respectively. These outbreaks were caused by the highly pathogenic field isolates that was also known as very virulent strains (vvIBDV) and capable of causing up to 100% mortality in specific-pathogen-free (SPF) chickens (11).

The incubation period of IBD is about two to three days. The infected chickens will then be having watery diarrhoea and become exhausted, prostrated, dehydrated, and ruffled feathers (12). Usually death follows at three days post infection. The flock mortality rate reaches the peak at day four, but will rapidly drop. The survivals, despite having the destructed bursas, will recover by five to seven days post infection (9). Nevertheless, because their bursa had been destructed by the virus, the recovered birds became immunosuppressed and susceptible to any opportunistic infection.

Outbreaks of IBD may not always be noticeable; particularly when the flocks' maternal antibodies were present or the involved IBDV strains were of low pathogenicity (9). The infected chickens, though may appear healthy, were indeed immunosuppressed and unresponsive to the costly vaccination programmes. Subclinical IBDV infection is not uncommon in the field and may prevail especially after the decline of passive immunity (9). On the other hand, acute outbreaks with high flock mortality rate usually suggested that vvIBDV strains are involved (9). And should the virus

persist in the farm premises and transmitted to the successive flocks, the clinical signs will appear earlier and gradually replaced by the subclinical forms (9). However, the farm still suffers from episodes of acute IBD outbreaks.

The causative agent is a bisegmented, double stranded RNA virus that belongs to the family Birnaviridae. IBDV is a non-enveloped icosahedral virus, approximately 58-60 nm in diameter (9). IBDV is endemic in most poultry producing areas of the world. The virus is highly stable and has a tendency to persist in the environment despite thorough cleaning and disinfections. The virus is highly stable to chemical and physical agents (12). In poultry houses the virus can remain viable for up to 60 days in the litter (13). There are two serotypes of IBDV: serotype 1 and 2. All viruses capable of causing disease in chickens belong to serotype 1; serotype 2 viruses may infect chickens and turkeys and are considered non-pathogenic for both species (14). Viruses of both serotypes of IBDV share common group antigens that can be detected by fluorescent antibody test (FAT) and enzyme linked immunosorbent assay (ELISA) (15). The caIBDV was the predominant strain until early 1980s (9). The vaIBDV which is antigenically different from the caIBDV was reported in the mid 1980s in USA (16). Snyder *et al*. (16) pointed out that this Variant strain is also different from the classical strain in that it results in severe bursal atrophy and also the vaccine produced from classical strain did not give full protection against the vaIBDV. In late 1980s; however, vvIBDV which caused an acute IBDV was reported from Europe with a high mortality in young chickens ranging from 21 to 35 days of age (11). 100% mortality was reported in susceptible chickens (17).

Along with strict hygiene management of poultry farms, vaccination with conventional live attenuated and inactivated viral vaccines has been used to prevent IBD. Furthermore, the control of IBD through depopulation of infected farms and disinfection of infected premises was practiced for so long but considered ineffective and costly (9). It was recorded that although identified more than 40 years ago, IBDV continues to be a major threat to commercial poultry all over the world (3; 9). Strict bio-security, together with the use of conventional inactivated and live IBDV vaccines to control IBD had been a success story until the early 1980s, when antigenic variants emerged. These changes in antigenicity and virulence made the task of controlling IBD by vaccination more challenging (3; 5; 17). Moreover, interference of maternally derived antibodies with vaccine uptake still remains a major problem in vaccination against IBD especially using live vaccines. Meeusen *et al.* (18) indicated that currently the market-available new technologies and next-generation vaccines in order to help prevent IBD more effectively. Therefore, the control and prevention of IBD is highlighted and discussed.

2. Control and Prevention of Infectious Bursal Disease

2.1 Exclusion or Eradication

IBDV is very resistance to the physical and chemical agents (19). The virus remains viable for at least 6 months in dry litter and more than 1 year in unused dry chicken houses (20). Its persistence in the environment, even after disinfection, makes the eradication in the affected countries seems unrealistic (9).

To prevent IBD, Maris (21) proposed several precautions such as practicing “all-in/all-out” farming methods; cleaning and disinfecting premises; and having a period of rest between depopulation and restocking (21). The use of 10% hydrogen peroxide as the microaerosol mist can inactivate IBDV particles (22); which is worthwhile to consider in the planning of the cleaning regime. In addition, several guidelines on the cleaning or disinfecting the IBDV-contaminated farm premises had been described: Before cleaning, all insects and pests (for example rats and mice) need to be eliminated. After removing and decomposing the old bedding and dung, all farm equipments are disassembled and relocated into a cleaning room outside the farm buildings. First, farm buildings are dry-cleaned. This is followed by washing with hot water (60 ^{o}C) and detergent at a pressure of 80 to 150 bar. The concentration of the disinfectants should be about 4 litres per 15m^2 (23). And before introducing the new chicks, second disinfection of the full premises is warranted. The feed that remained from the previous flocks must never be reused (9).

2.2 Genetic Selection for Resistance

The susceptibility of the host to various poultry pathogens depends mainly on its genetic makeup (24). Resistance to IBDV infection could be breed-dependent, and crosses between resistant and susceptible lines had indicated the resistance is a dominant hereditary phenotype (9). Light breeds of chickens may have higher mortality rates than the heavier breeds (25), but inoculating IBDV in other avian species failed to cause the disease (26). Unfortunately, the genes that confer the resistance against IBDV are yet identified and it is not a common practice to selectively breed the resistance lines (25).

2.3 Vaccination

Vaccination is the principal method of controlling viral disease in commercial poultry worldwide (27), but never the substitute for good animal husbandry and hygiene practices. The success of vaccination depends on the choice of vaccine strain, vaccination schedule, and the strains of the field isolate (9). In the field, outbreaks of IBD have been controlled by vaccination practices (28).

The assorted IBDV strains with diversified antigenicity (29) have complicated the vaccination programmes. Take, for instance, the inactivated vaccines prepared from the vvIBDV strain may protect against the classical strain (STC isolate) but provided no protection against the variant strain (IN isolate) (30). In addition, antibodies against serotype 2 strain do not protect the birds from a virulent serotype 1 challenge (31). Therefore, vaccination against an IBDV strain may not protect the chickens against other strains challenges. Several types of IBD vaccines are available, namely the live, inactivated, and recombinant vaccines. Generally, recombinant vaccines may consist of the IBDV antigenic proteins (usually VP2) that had been expressed in different expression systems. DNA vaccine, on the other hand, may contain the genetic sequence (one or more genes) of IBDV.

In practice, the attenuated live virus and oil-emulsion inactivated virus vaccines are used. The general principles regarding the choice and use of the IBD vaccines remained valid (9). And the standard requirements for preparing IBD vaccine were also described (32); however, these requirements may be too idealistic and difficult, if not impossible, to achieve. In vitro antigen quantification had been reported as an alternative potency assay to measure the efficacy of IBD vaccine (using VP3) because it is faster, more economical, and can avoid the used of experimental animals (33). The ideal vaccine must not cause the disease or bursal lesions, must not be immunosuppressive or excreted, and must confer long-lasting immunity even in the birds that have high maternal immunity. Unfortunately, such ideal vaccine is yet to be found (26).

Early vaccination at 7 days was reported to be superior to vaccination the birds at 14 or 28 days for better antibody response and protection against mortality and bursal lesions (34). Chickens vaccinated with IBDV in early life and revaccinated with an inactivated, oil-adjuvant IBD vaccine at 18 weeks of age produced and maintained high levels of virus-neutralizing antibody through 10 months of lay (35). The route of vaccination, such as oral followed by parenteral administrations of IBDV antigen had been reported to induce an enhanced antibody response in chickens (36).

2.3.1 Live Virus Vaccines

Live virus vaccines are generally derived from the serial passages in embryonated eggs (9). In general, the live IBDV vaccines in use by the poultry industry have been attenuated by serial passage in tissue culture, eggs or embryo-derived tissues, with the aim of maintaining the immune response induced by the parent virus whilst attenuating the ability of the vaccine virus to cause clinical disease or significant immunosuppression (37).

The degree of attenuation of the vaccine strains can be classified as mild, intermediate, and hot; depending on its ability to cause the varying degree of histological lesions (38). Although serotype 1 vaccine strains cause no mortality, it still causes different degrees of bursal lesions that range from mild to moderate or even severe (9). The higher the virulence of the vaccine virus strain, the more severe damage of the bursal lymphocytes resulted (39). Nonetheless, as it should be, the lesion caused by the vaccine strain is less severe than the field strain (40). The mild strain is mainly used in the breeder vaccination programme. Given the mild strain subjects to the maternal antibody interference, it is therefore usually used between the fourth and eight week of age, depending on whether the grandparent birds have or have not been vaccinated with oil-emulsion inactivated vaccine before lay (9). Intermediate vaccines are used for broiler and pullet vaccination (41), and sometimes to breeder chicks when the flocks are at risk of early challenge of highly pathogenic strains. Day-old vaccination using intermediate vaccine may protect the chicks that have insufficient maternal antibody (9). Besides, early vaccination will spread the vaccine virus in the farm premises and provides indirect vaccination to the other susceptible chicks (9). In high-risks farms, two vaccinations are generally practice. The time of vaccination depends on the flocks' maternal antibody titres. Route of vaccination is usually through drinking water, although nebulisation could also be used (9). To achieve higher maternal antibody in the progeny, vaccination of broiler breeders with live IBD vaccine by the oral route is better than the intramuscular injection (42). Meanwhile, vaccination of parent chickens with a commercial live IBD vaccine under field conditions at varying ages and by different routes may result in the variable susceptibility to the disease in their chicks (43).

In ovo vaccination with a mixture of vaccines against IBD and Marek's disease protects the hatched chicks against both diseases (44) without inhibiting individual viral agents on humoral and cellular immune competence (45). Moreover the use of a multivalent *in ovo* vaccine (comprised of IBDV, Marek's disease virus, and a recombinant fowl poxvirus vector that contained HN and F genes of Newcastle disease virus (ND)) was reported successful in field conditions (46). *In ovo* vaccination using IBDV alone could resisted the challenges with pathogenic IBDV at 4, 6, 8, and 10 weeks of age (47). Notwithstanding with the above findings, other scientists reported that although *in ovo* IBD vaccination may protect SPF chickens from IBDV challenge, the protection in commercial chickens was incomplete after the challenge - evidence by the presence of bursal lesions (48). *In ovo* vaccination may also reduce the immune response to ND vaccination in SPF chickens, but similar phenomenon was not observed in commercial chickens (48). Other found that *in ovo* vaccines may cause significant microscopic lesions in the bursa of Fabricius at 1 and 3 wk post-hatch (49).

In ovo vaccination with "antibody-mixed live vaccine" provides an alternative mean of vaccination, in which this practice may avoid the interference from the maternal antibodies and protect the chickens against IBD (50). Whitfill *et al*. (51) developed this type of IBD vaccine by mixing the anti-IBDV antibody with the virus particles itself (52) (referred as "antibody-mixed live vaccine"). The vaccine was administered through *in ovo* route to the SPF embryos and was reported to be safer and more potent than the conventional IBD vaccine because it delayed the appearance of

bursal lesions, produced higher geometric mean antibody titers against IBDV, generated protective immunity against challenge, and produced no early mortality (53). The working mechanism of "antibody-mixed live vaccine" was thought to be related to its specific cellular interaction with the follicular dendritic cells in spleen and bursa (54). The disadvantages of *in ovo* vaccination using "antibody-mixed live vaccine" might be the transient bursal destruction, observed both in SPF and commercial broilers (55). Some reported the vaccine may cause bursal atrophy (56, 57) and cell-mediated immunosuppression (57).

The "antibody-mixed live vaccine" had been given various names and may lead to confusion, these names were: "IBDV–bursal disease antibody (IBDV-BDA) vaccine" (50; 55), "BDA-IBDV" (53), "IBDV immune complex vaccine (ICX)" (56), "*in ovo* complex vaccine" (39), and "antibody-coated IBDV vaccine" (58). Here, the author has no intention to create another new name, but the term "antibody-mixed live vaccine" is simple enough and yet contains the essence of what it shall mean.

In summary, live IBD vaccines must not cause serious bursal lesions and immunosuppression to be compatible with other avian vaccines. Registration procedures for the IBD vaccines stated that candidate vaccines must not interfere with other vaccinations and will not revert to virulence in the course of serial passages in three- to six-week-old SPF chickens (9).

2.3.2 Inactivated Vaccines

Inactivated vaccines are usually used in the breeder hens for them to pass down high, uniform, and persistent antibody titres to the progeny (59; 60; 61). For the vaccination to be effective, the hens must be previously vaccinated with a live virus or had been exposed to the virus in the farm. Inactivated vaccines are administered to the layers through subcutaneous or the intramuscular route at sixteen- to twenty-week-old. In this way, the chicks will have the protective maternal antibodies up to thirty days (62; 63; 64; 65). However, the chicks will not be protected from the challenge from the highly pathogenic IBDV strains at later age (60; 64). Inactivated vaccine is usually prepared from the bursal homogenates of infected chickens or from viral cultures on embryonated eggs or fibroblast cells; where the virus is then inactivated by formaldehyde and various alkylating agents like binaryethylenimine (BEL) and betapropiolactone and prepared as the oil emulsions (9). Physical means such as high hydrostatic pressure can also produce inactivated vaccine by dissociating the virus particles into subunits while preserving its immunogenicity (66).

2.3.3 Recombinant and DNA Vaccines

Infectious bursal disease virus proteins expressed in other prokaryotic systems can serve as IBD recombinant vaccine. The recombinant IBDV protein will be a more effective vaccine if it precisely mimic the authentic molecular structure of the viral protein (67). Structural proteins of IBDV had been expressed in the baculovirus expression system. The baculovirus-expressed protein induces immunological response (68) and protects the chickens from IBDV challenge (69). However, the protection is incomplete, evidence by the presence of bursal damage after IBDV challenge (70). In comparison with virus-like particles (VLP), VPX tubules, and polyprotein-derived mix structures, the baculovirus-expressed VP2 capsids elicit stronger immune response (67). Improved technology for producing recombinant IBDV protein using baculovirus expression system had also been documented (71).

Reports indicated that VP2 had also been expressed in other expression vectors such as the herpesvirus (72), Marek's disease virus (73), fowl adenovirus (Sheppard *et al*., 1998), fowlpox virus (73; 74), and Semliki Forest virus (75); in which they may serve as recombinant IBD vaccines. Recombinant fowlpox vaccine protects the chickens from the IBDV-induced bursal damage but its efficacy depends on the titre of the challenge virus and the chicken genotype (76). In addition, the effective application of recombinant fowlpox (VP2) vaccine may be restricted to the wing web and parenteral routes of inoculation (77). In eukaryotic expression system, VP2 expressed in the yeast confer passive protection against IBD (78); probably because the multimeric forms yeast-derived VP2 were highly immunogenic (79). Expressions of VP2 in *E. coli* are not immunogenic (79). Aside from single type of recombinant vaccine, the dual-viral vector approach – an approach that uses Marek's disease and Fowlpox viruses that express vvIBDV host-protective antigen may serve as a quick and safe method in inducing strong and long-lasting protective immunity against vvIBDV (7).

Deoxyribonucleic Acid vaccine could provide efficacious protection for chickens against IBDV infection (80). Effective DNA vaccine included the VP2 gene in the plasmid DNA (81). Transcutaneous plasmid-dimethylsulfoxide (DMSO) delivery technique for avian nucleic acid immunization had been described (82). It was pointed out that DMSO enhances liposome-mediated transfection of nucleic acid in chicken macrophage cells and this phenomenon was exploited for the transcutaneous delivery of naked DNA through the intact skin of the chickens. DNA-based IBD vaccine had been delivered using this technique and the chickens were protected against IBD (86% survival) (82). Recombinant vaccines offer several advantages over other types of vaccines such as the absence of residual pathogenicity, low sensitivity to maternal antibodies, low risk of selection of mutants, the possibility to administered through *in ovo* route, and may enable one to distinguish between the infected and vaccinated animals (74; 83; 84; 85). Although these vaccines are said to be available in the market (18), nevertheless, the effectiveness of recombinant vaccines should be validated at the level of commercial poultry level.

3. Vaccination Failures

The widespread vaccination had led to the increased virulence of IBDV (37); but apart from the increased virulence, various causes must be considered to deal with vaccination failure. The trivial causes must be first rule out: inappropriate storage of vaccine, inappropriate vaccination techniques, should have read the expiry date and recommended doses, and should have used distilled water in diluting the freeze-dried live vaccines. If vaccine is to be administered through drinking water, the flocks should be water-deprived for two to three hours before distributing the vaccine into the water. Addition of milk powder at a concentration of 2 g per litre stabilizes the water-soluble vaccine (9).

One of the most frequent causes of vaccination failure is the interference of the maternal antibody. Therefore the immune status of the chicks and the vaccination protocol of the parent stocks should be determined in planning a vaccination programme (9). The optimum vaccination time can be estimated by titrating the maternal IBDV antibodies of 1-day-old chicks using ELISA or agar gel precipitation test (AGPT) (86). The average level of maternal antibody to IBD in day-old layer strain chicks is approximately 45% of the antibody titre as in their respective dam (87). Whether the chicks vaccinated with inactivated vaccine could be protected from the disease depending on the virulence of the challenge IBDV strains and the strain that being used in the vaccine (88). In addition, one should note that vaccine prepared from the classical strains do not give full protection against variant and very virulent strains (89).

4. Anti-viral Drugs

Apart from producing the myriad types of IBD vaccines, other scientists are in search of alternative ways to fight against the disease. For example, by feeding *Azadirachta indica* (Neem) dry leaves powder to the IBDV-infected birds, scientist found the bird's humoral and cell-mediated immune response were improved (90). Supplementation of ascorbic acid at 1,000 ppm in the diet is beneficial to the chickens that are vaccinated against IBD (91). This is probably because ascorbic acid has ameliorated the immunosuppression caused by IBDV vaccination and thus improved the humoral and cellular immune responses of the vaccinated birds (92). Moreover, ascorbic acid supplemented birds have higher body weight gains in comparison with the non-supplemented group (91).

Other suggested that feeding crude thymus extract to the IBD-vaccinated chicks may improve the vaccination effectiveness because this practice could improve the body weight gain and conferred better protection against IBDV challenge (93). Virus neutralization factor (VNF) is a class of non-specific antiviral agents produced in vivo in chickens in response to viral infection and can directly inactivate the IBDV particles (94). Meanwhile, inoculating concentrated anti-IBDV immunoglobulin extracted from the egg yolk into SPF embryonated eggs may produce chicks with passive immunity and protected against IBD (95).

The recombinant interferon alpha, which has antiviral effect, has shown to suppressed IBDV plaque formation in a dose-dependent manner and ameliorated IBDV and ND virus infection in both SPF and commercial chickens (96). The effect of the interferon therapy, while depending on the route of administration, is more obvious in commercial chickens than in SPF chickens (96).

5. Conclusions and Recommendations

In conclusion, choices to control IBD seem limited. While the emphasis is on prevention rather than cure, there is not much one can do with the infected flocks once IBD outbreak had occurred in the farm. In addition, eliminating the sturdy and persistence IBDV particles from the farm is by no means an easy task. In practice, there is little value in treating the IBDV-infected birds because of the incurred cost. And even if the young birds do recover from IBD, their bursas had already been destroyed and therefore vulnerable to other infections. Culling the infected chickens and prevent other flocks from being infected is a sensible approach, though costly. In other words, now, vaccination remains as the principal method to curb the disease. However, even with stringent vaccination practices, the farm is still not free from the threat of IBD because vaccination against an IBDV strain may not fully protect the birds from being infected by other strains. Although the use of recombinant and DNA based vaccines has added advantages over the conventional inactivated and lives vaccines protection level of 100% is yet to be achieved. Supplementation of IBD vaccinated chickens through feeding *Azadirachta indica* (Neem) dry leaves powder and also supplementation of ascorbic acid were believed to enhance the immune response of infected chickens but this is still under experimental conditions and has to be practiced and validated. The use of anti-viral drugs such as recombinant interferon alpha, Ribozyme R4 has been tried by researchers but the result is still guarded and needs further investigation. Therefore, in depth studies should be done on investigation of the epidemiology of these viral diseases. The effectiveness of the already developed new technologies and next-generation vaccines should be ascertained under commercial poultry level. Knowledge on the use of vaccines against this disease should be exploited, so as to have cost effective prevention methods. Furthermore, attempts should emphasize on the identification of local viral strains present in the field to design cost effective vaccine

as well as for selecting a vaccine judiciously and for formulating effective control and preventive strategies against the disease in the country.

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