

## INFECTIOUS BURSAL DISEASE

### DESCRIPTION OF THE DISEASE

Infectious bursal disease (IBD) was discovered in 1957 in Gumboro, Delaware, USA. As a result, the disease is often referred to as Gumboro. Not long after IBD was first reported, it was being recognized in poultry populations throughout the world.

IBD is caused by a virus classified as a birnavirus. There are two basic serotypes, I and II. Most isolates of serotype I are of chicken origin and most isolates of serotype II are of turkey origin. Within each serotype, a variation in antigenic structure exists. Variants within serotype I have been studied extensively since their discovery in 1985.

When IBD was first recognized, it was characterized by whitish or watery diarrhea, anorexia, depression, trembling, weakness, and death. This clinical IBD was generally seen in birds between three and eight weeks of age. The course of the disease runs approximately 10 days. Mortality usually ranges from 0-30 percent. Field reports suggest leghorns to be more susceptible to IBD.

Subclinical IBD was later recognized and is a greater problem in commercial poultry than the clinical disease. It is generally seen in birds less than three weeks of age. No clinical signs are generally seen. This early infection results in a lymphoid depletion of the bursa of fabricius. The bird is immunologically crippled and unable to fully respond to vaccine or field virus. In addition, the bird may be susceptible to agents that are not normally pathogenic (adenovirus, clostridial infections).

In susceptible chickens, damage from IBD can be seen within two days of exposure to virulent virus. Upon ingestion, the virus reaches the bursa via the blood stream or through the opening that exists between the gut and bursa. Upon entering the bursa, extensive replication occurs. Initially, the bursa swells (3 days post-exposure) and then begins to atrophy (7-10 days). The bursal wall becomes thin and the internal folds may be seen through the wall. Occasionally, hemorrhages may occur within the bursa and the birds may pass blood with their feces. Variations in this progression of events have been noted. Variant strains of serotype I, isolated in 1985, in the Delmarva area (USA), have been shown to cause bursal atrophy in as little as three days post-infection.

The bursa is responsible for taking embryonic stem cells, received from the yolk sac, and turning them into competent B-lymphocytes. From day 8 to 14 of incubation, these stem cells enter the bursa. Once inside the bursa, these stem cells begin the maturing process to B-lymphocytes. At day 17 of incubation, those B-lymphocytes that have matured begin to migrate, through the blood stream to secondary lymphoid organs. Migration of B-cells is also seen in the thymus. Secondary lymphoid organs include the spleen, hardarian gland, cecal

tonsil, and gut-associated lymphoid tissue. This migration of B-lymphocytes peaks at three weeks and is complete by sexual maturity.

IBD virus is cytopathic to only certain B-lymphocytes. The highest concentration of these specific B-lymphocytes is found in the bursa. Destruction from IBD field virus results in an incomplete seeding in the secondary lymphoid tissue. As a result, the bird is immunocompromised and not capable of responding to other pathogenic agents or vaccines

IBD virus can be found throughout the world. The occurrence of clinical IBD is relatively low compared to the prevalence of subclinical Gumboro. The IBD virus is very resistant to common disinfectants and has been found in lesser meal worms, mites, and mosquitoes. These facts correlate with field experience of reoccurring IBD problems on a farm, despite clean up efforts.

Infection with IBD results in a strong antibody response. Even in birds that have been compromised by an earlier IBD exposure go on to produce high levels of antibodies against IBD. But, response to other viruses will be negatively affected.

## **FACTORS TO CONSIDER BEFORE DESIGNING AN IBD VACCINATION PROGRAM**

The more information acquired prior to designing a vaccination program, the better the chance of a successful program. Some of these factors include:

1. Virus quantity in the environment
2. Characteristics of the virus in the environment
3. Level of maternal immunity
4. Genetic resistance
5. Mixing numerous breeder flock progeny

### **Virus Quantity**

IBD is a stable virus and is resistant to most common disinfectants. Phenolic disinfectants have had some efficacy as well as formaldehyde fumigation. Formaldehyde fumigation has the advantage of being able to permeate into areas that are not accessible to liquid disinfectants. Once a house is seeded with IBD, it should be considered; thereafter, to be a house problem.

Increasing the 'down time' between growouts has also been reported to reduce the IBD challenge somewhat. By allowing the house to remain empty for 2-3 weeks, removing old litter, and washing and disinfecting IBD challenge has been less evident.

Brooding on paper has met with varied success. In some operations, it has helped reduce early exposure to Marek's Disease and IBD. This is especially true where built up litter has been used. A disadvantage of putting down paper is it tends to trap moisture resulting in high levels of ammonia and eventually caked litter.

## **Virus Characteristics**

IBD viruses are not all the same. The field IBD viruses may vary in their virulence, immunogenicity, and antigenic make up. Virulence refers to a viruses' ability to enter the bird and destroy target cells and tissue (B-cells and bursa in the case of IBD). This variation has been demonstrated most frequently by determining a viruses' ability to infect a bird possessing varying amounts of maternal antibody (MA). The more virulent the viruses are, the more capable they are of establishing themselves in the face of high MA. Once they are established, seroconversion is noted.

## **Maternal Immunity**

Vaccination of breeder hens with vaccine containing IBD has become widespread practice throughout the world. This is done because of the ability of the hen to transfer antibodies against IBD from her bloodstream to the chick's yolk sac. The transfer of antibodies, from hen to chicks, is an efficient process.

Maternal antibodies (MA) are efficient neutralizers of IBD. This passive protection, provided by MAs, prevents bursal atrophy and immunosuppression. These two criteria (bursal atrophy and immunosuppression) need to be distinguished, because bursal atrophy may be seen without immunosuppression. However, the levels of MA's necessary to neutralize IBD vary with the invasiveness and pathogenicity of the virus strain. In practical terms, if a "hot" (invasive, pathogenic) IBD challenge is present, higher MA levels and/or an effective progeny vaccination program will provide the desired protection.

## **Vaccination**

The goal of vaccinating for IBD is prevention of subclinical and clinical Gumboro and the economic aspects of each. In reality, vaccinating for IBD is not 'all or nothing'. Instead of preventing infection of IBD, we are attempting to minimize the effects of its infection. Due to our management systems and the biology of the virus itself, prevention is often impractical.

Vaccination for IBD is approached by using two basic concepts.

1. High levels of maternal antibodies are protective
2. Effective vaccination in the field induces protection starting before 5 days post-vaccination.

As has been discussed, maternal antibodies make vaccinating in the field difficult. Because of this difficulty, there are a variety of vaccines available. Knowledge of these vaccines is essential to effectively design a vaccination program for IBD.

## **MODIFIED LIVE VACCINES**

There are two things to consider when examining a modified live vaccine. These include:

1. Invasiveness - addresses the ability of the virus to replicate in the face of maternal antibody.
2. Spectrum of antigenic content - addresses original seed strain and vaccine preparation technique.

A vaccine virus' ability to replicate in the face of maternal antibodies allows live vaccine to be categorized into three groups: mild, intermediate, and strong. These were developed at different times in the history of IBD research and for specific reasons. The initial vaccines for IBD were of the strong variety. These were often used in breeder programs to induce high levels of circulating antibodies. However, when given to a young bird with moderate (100-200 on serum neutralization (SN)) or low levels (<100 on SN) of maternal antibodies, these vaccines could cause extensive bursal atrophy resulting in immunosuppression. Mild vaccines were developed to be used in young birds. These vaccines are not immunosuppressive even when used in birds having no maternal antibodies. However, they are easily neutralized by moderate and high levels (>100 on SN) of MA. As breeder programs developed (including the use of adjuvanted killed vaccines), higher levels of maternal antibodies were generated in progeny. This reduces the effectiveness of these mild vaccines.

Intermediate strength vaccines were developed to overcome the inadequacies of the mild vaccines. They are capable of establishing immunity in birds with moderate levels of maternal antibodies (100-200 on SN). These vaccines will cause some bursal atrophy in MA negative birds. However, research and field experience has shown them not to be immunosuppressive. The following table summarizes these characteristics.

<u>Type of Vaccine</u>	<u>Ability to Overcome Moderate MA</u>	<u>Immunosuppressive In Birds With Low To Moderate MA</u>
Mild	-	-
Intermediate	+	-
Strong	+	+

### **SPECTRUM OF ANTIGENIC CONTENT**

The spectrum of antigenic content of a live IBD vaccine is a newer characteristic to consider. It has become evident that a variation in antigenic content exists in IBD field isolates in some areas. There is also a variation in antigenic content within vaccines. This variation is dependent upon the original seed strain-selected for the vaccine as well as the technique used in the production process.

Techniques used in manufacturing IBD vaccines may involve some manipulation of the original seed strain. Some manipulations may limit the variation in antigenic content which naturally exists within the vaccine seed strain. An example of this is cloning.

## **KILLED VACCINES**

Inactivated IBD vaccines are used in broiler breeders throughout the world. They differ in some of the same ways as live vaccines. Their efficacy depends upon spectrum of antigens they contain. This is related to the original seed strain and the manipulation of that seed strain. The wider range of antigenic spectrum, the increased chance that the antibodies passed to progeny will neutralize the existing field challenge viruses.

There are three basic ways antigen for killed vaccines are grown. These include tissue culture origin (TCO), chick embryo origin (CEO) and bursal tissue derived (BTO). BTO produces the highest quality antigen and the best immune response. This is followed by CEO and then TC, being the least effective.

## **APPLICATION TECHNIQUES OF IBD VACCINE**

Commercially available IBD vaccines vary on recommended application method. Possible routes for application of live vaccines include subcutaneous, eye drop/nasal drop, spray, and water. Injectable oil-emulsion products may be given subcutaneously or intramuscularly.

Live vaccines must be given in a way in which the virus will reach the bursa where it will multiply and initiate an immune response. When given subcutaneously, the vaccine virus enters the blood stream and is transported to the bursa to replication. This same scenario is also seen by eye drop/nasal drop, spray and water methods. Eye drop/nasal drop and spray first are inhaled before entering the blood stream. IBD vaccine given via the drinking water (as well as any virus swallowed in spray and eyedrop/nasal drop applications) reaches - the bursa two ways. As it is swallowed, some virus is absorbed through the gut lining into the blood stream. Virus that stays within the gut can enter the bursa through the communication which exists between the bursa and gut.

All of these methods of application are capable of working. However, it is best to follow the manufacturers recommendations for each product. The application routes on the label have been proven safe and efficacious.

Inactivated IBD vaccines are generally licensed with both subcutaneous and intramuscular routes approved. Administration should be done carefully, as with all injections.

## **VACCINATION**

Effective vaccination for IBD can be divided into four categories:

1. Protecting the developing bursa (broilers, breeders, layers)
2. Preventing clinical disease (broilers, breeders, layers)
3. Priming (breeders)
4. Boosting (breeders)

## **PROTECTING THE DEVELOPING BURSA**

The bursa needs to be protected from the immunosuppressive effects of IBD. This is accomplished by preventing significant bursal atrophy. Immunosuppression resulting from IBDV is age, dose, and strain related. The younger the bird, the more extensive the immunosuppression. Protection from bursal atrophy for the first 14 days of life prevents any permanent immunosuppression from occurring.

The higher the dose, the more permanent the immunosuppression. Birds exposed to a high dose of very pathogenic IBD virus may be permanently immunosuppressed. Birds exposed to a lower dose of the same IBD may not respond properly to the initial Newcastle disease (NDV) vaccination but when vaccinated later are capable of responding well. In other words, the immunosuppression was temporary.

## **APPROACH**

To minimize the immunosuppressive effects of IBDV, each of these points (age, dose, and strains) should be addressed.

### **Age**

Protection to the very young can be achieved through maternal antibodies passed from the breeder hen to her progeny. This requires an aggressive and well implemented breeder vaccination program.

Vaccination of the very young chick itself may not be successful. Onset of protection after vaccination is between three and five days. When a bird lacking MA protection is introduced to a pathogenic field strain of IBD, the damage will be done in 24-48 hours. Field experience with vaccination in the very young (within the first week of life) has yielded variable results due to MA interference and the points mentioned above.

### **Dose**

The dose of pathogenic IBDV, the young chick receives can be reduced through management. The management practices that have helped reduce the quantity of IBD field challenge include:

1. Cleaning and disinfecting between growouts (including removal of old litter).
2. Allowing the house to remain empty at least two weeks between growouts.
3. Brooding paper placed prior to housing new chicks

### **Strain**

Attempt to replace the field strain of IBDV with a vaccine strain that is not immunosuppressive. Once IBD invades the bursa, the virus is shed into the environment in large numbers. This multiplier effect will be seen with either the field strain or the vaccine strain. In field situations where vaccines have been used for several consecutive growouts, a reduction in strain pathogenicity has been experienced. Although not scientifically proven, the vaccine strain seems to replace the field strain. With this in mind, vaccination programs should be evaluated over three growouts.

## **PREVENTING CLINICAL DISEASE**

Effective vaccination, avoiding MA interference, will help prevent clinical IBD. Clinical IBD is typically seen between three and six weeks of age. This also coincides with the time period where MA are rarely present. The immune response of the chick must be stimulated as the passive protection (MA) is metabolized. By using (estimating - where titers are not available) the breeder or chick titer and a MA half-life of four days, the timing of initial vaccination may be estimated.

MA tend to vary within population. This is due to the breeder hen variation as well as progeny from several breeder flocks are often grown together. For this reason, it is recommended that the initial vaccination be followed with a second vaccination 4-10 days later.

Clinical IBD is rarely seen after 8 weeks of age. One effective vaccination is sufficient to protect birds for this time period.

## **PRIMING**

Priming is a term that refers to preparing the immune system for a killed vaccine. This involves introduction of live vaccine or field challenge a number of times so the bird responds and makes memory cells to IBDV. By doing this, the optimal response is seen from the administration of the inactivated antigen.

The early vaccinations serve as primers, although they may not be enough to create an optimal amount of memory cells. The bird responds to the early vaccination by creating memory cells and plasma cells and eventually, antibodies. The more effective and complete these early vaccinations are, the more complete the priming. In most situations, this is not considered adequate.

Field challenge may be suggested as the "best primer". In actuality, it may well be; however, it is unreliable. If relying on field challenge, a third of the flock may be well primed, a third of the flock may be moderately primed, and a third of the flock may be poorly primed. This results in an uneven boosting with the inactivated vaccine.

## **BOOSTING**

Boosting is the term commonly associated with the administration of a final vaccination prior to the onset of lay. This is done to increase the circulating antibody in the hen. This, in turn, raises the MA passed to her progeny. Both inactivated and live products have been used for this purpose, with inactivated being the more popular.

Live boosting was popular prior to the development of inactivated vaccines. The strongest product available was often used at 3X dose. The use of a live vaccine in an older bird will result in a boost; however, large variations are often seen. These variations resulted in progeny becoming susceptible to field challenge from as early as a few days of age out to 14 days of age.

The use of inactivated IBD vaccines gave a higher titer as well as decreased the amount of variation seen between birds' responses. It is difficult to quantitate how much higher, due to the many variables involved, but progeny were protected for as many as 10 days longer. Progeny from breeders that are properly primed and boosted with an inactivated IBDV vaccine are generally protected from 7 to 21 days within a given operation.

This variation may be 14-21 days for younger breeder hens (<40 weeks) and 7-14 days for older breeder hens (>40 weeks).

As breeders age, the titer to IBDV deteriorates slowly. Again, variations exist, but an estimate of 1 log base 10 every 8-10 weeks may be a useful tool. Due to this aging and the initial variations which existed, mixing progeny from old and new breeder flocks complicates IBD field vaccination.

Modifications in boosting programs have been used in high challenge areas. In some places, two inactivated boosters are administered 6 weeks apart with the last being 4 weeks prior to egg production. This is done to reduce the variation within the flock even further. A second modification is to administer another inactivated boost while the hens are in mid-production. This reduces the variation seen in MA titers from progeny of old and young flocks. There are places in the world where both of these modifications are practiced.

The most common mistakes seen in breeder IBD programs stem from inadequate priming and poor injection technique with the booster. Priming needs to set the foundation for the booster. If it is not solidly in place, disappointing results will be seen thereafter. Administration of inactivated vaccines, in general, must be done carefully and properly placed within the muscle of the breast or subcutaneously along the neck. (Follow label directions.)

There are many inactivated products which contain multiple antigens, including IBD. These products, if they have been USDA licensed, have had to pass efficacy tests concerning each antigen they contain. Therefore, they are safe and effective. This does not mean mixing two products within a syringe produces the same efficacy.

## **HOW TO MEASURE RESULTS**

### **Performance**

An IBD vaccination program is best evaluated by examining overall performance. This must include livability, feed conversion, weights, and condemnations. An effective program, when instituted on a problem farm, is capable of making improvements in all the above categories.

### **Serology**

Serology for IBDV has been done with several tests. These include mainly the enzyme linked immunosorbent assay (ELISA) and virus neutralization (VN) tests. The two have been found to correlate to some degree. It has been seen that VN tend to correlate better with protection than ELISA. Quantitative agar gel precipitin (QAGP) test has also been used. However, the QAGP is not as sensitive as the other two.

Serology for IBD must be examined critically. Monitoring titers to IBD is useful in estimating when MA's reach a level that vaccination can be done effectively. This has been mentioned previously. IBD serological tests are also useful in determining the virulence of field challenge. Monitoring MAs throughout their decline, seroconversion will eventually be seen from field strain. If this happens while MAs are still relatively high (> 200 on SN), the field challenge should be considered strong. The majority of field strains seem to be intermediate strength and above.

IBD serology may be used in evaluating the priming vaccinations in breeders. Most companies consider titers of 1:150 to 1:200 (on a VN test) appropriate priming. In some areas, the cost of doing serology is prohibitive. In these situations, an "assurance primer" should always be given at 10-12 weeks.

Breeder hen titers are often taken periodically to assure the desired MA's in progeny. Most vaccination programs using an inactivated vaccine put out progeny with MA lasting from 7 to 21 days depending on age, breed, environment, and other factors.

### **Examination of Bursal Atrophy**

Examination of bursae as a bird ages is a useful, but often confusing, parameter to examine. Bursal atrophy may be done by gross examination, bursa to body weight ratio or histologic bursal score. Gross examination of the bursa is very subjective. Experienced service people and flock owners commonly "have an idea" that the bursal size is abnormal. Examination of the bursa should be done during post mortems but future decisions should only be made after large numbers of birds have been examined (as well as using a more exacting measurement tool, if possible). Examination of only dead or cull birds must be taken in context. A bird may have atrophied bursa from a number of other conditions including an excessive amount of stress, Marek's disease, and aflatoxin.

### **Histologic Bursal Scores**

A third method of quantitating bursa damage is by histologic examination. This may then be quantitated on a 0 to 4 scale with 4 being the worst. A general scoring criteria is listed below.

#### Bursal Lesion Scoring System

<u>Level of Severity</u>	<u>Description of Lesions</u>
0	No lesions
1	Mild, scattered cell depletion in a few follicles
1.5	25% of follicles are depleted of lymphocytes
2	Moderate, 1/2 of the follicles have atrophy or depletion of cells
2.5	75 of follicles are depleted of lymphocytes
3	Diffuse, atrophy of all follicles or depletion of cells in all follicles
4	Acute inflammation and acute necrosis typical Of IBD

The scores assigned to particular bursa may vary according to pathologist doing the examination.

## **Interpreting Bursal Size**

Correlating bursal size to performance at processing has not proven to be useful. The important point seems to be when the bursal damage appeared and what caused this damage. If the damage to the bursa was done prior to two weeks of age, the birds may be immunosuppressed. If the bursal damage was done at five weeks on top of a vaccination, there may be no effect from challenge at all. The point that becomes evident here is in strong challenges, bursal damage may not be prevented by even the best vaccination program. However, the birds process better and clinical IBD is not seen. Correlating the time of bursal damage by field challenge with performance would be a more useful tool.

Vaccines cause some bursal damage. In order to make intermediate vaccines effective in the face of some MA, they had to be invasive. They also must not be immunosuppressive when given to birds with no MA. The intermediate vaccines are proven non-immunosuppressive by vaccination with Newcastle vaccine following IBD vaccination and comparing the resulting titer with the titer of birds not given the IBD vaccine. However, bursal damage will be seen.

There have been three parameters mentioned in evaluating an IBD vaccination program: Performance, Serology, and Bursal Size. By far, the most meaningful is performance. This remains the bottom line. As indicated, performance of a vaccine should be done over at least three growouts.