

# Infectious Bursal Disease (IBD): Causative Agent, Diagnosis and Prevention

## Background:

IBD or Gumboro is an old disease and has been described by scientists all over the world. It is caused by a birnavirus; which targets the bursa of Fabricius – the primary organ involved in the development of the chicken's immune system. Most of the economic devastation associated with IBD is due to its immunosuppressive effects that lead to poor vaccination response, secondary bacterial, viral and protozoan infections and poor performance. The virus is now recognized in every poultry-producing country in the world. First diagnosed in 1962, the virus has since then changed and manifested itself in different forms that made it even a bigger threat to the industry.

## The Classic Strain:

### 1. Occurrence and Clinical signs:

IBD occurs in both layer and broiler birds and although it has been found in turkeys, the birds do not show infection. Morbidity can go from 10% to as high as 90% and mortality seldom exceed 3%. Layer type birds typically are affected more severely than meat type birds. Birds that are 3-6 weeks old are the most susceptible. Occurrence in younger birds is usually asymptomatic. Some studies have shown that age of infection is directly related to the degree of immunosuppression. Ivanyi and Morris (1976) demonstrated no immunosuppressive response after 3 weeks of infection despite the manifestation of a clinical disease.

The virus is highly contagious and spread by direct contact between infected and susceptible birds. It can also be transmitted by equipment, people, air, vehicle etc. Mice and darkling beetles can carry the organism for a very long time. IBD does not transmit from hen to progeny.

### 2. Gross and Microscopic Lesions:

- a. Ecchymotic hemorrhages in muscles and fascia of the breast and thighs due to the impairment of the clotting mechanism.
- b. Hemorrhages on the mucosa at the junction of the proventriculus and gizzard.
- c. Kidneys are enlarged and urates are accumulated in the tubules.
- d. Spleen is enlarged.
- e. The bursa of Fabricius is the main organ affected. The size is doubled 4 days post-infection and shows paleness with straw colored transudate. Sectioning of the bursa would demonstrate hemorrhages in the follicles as well as exudates. From the 5th day post-infection, the bursa will start receding in size until it is about 1/3 the size of an unaffected bursa.
- f. The IBD virus produces lesions in the bursa of Fabricius, spleen, thymus, cecal tonsils, gut-associated-lymphoid tissue (GALT), Harderian gland, and

Affected birds can start shedding the virus 24 hours post-infection. It takes about 2 to 4 days for the disease to occur. Clinical signs are severe depression; vent picking, presence of urate stains on the vent, diarrhea, dehydration loss of appetite and elevated water consumption. These signs can vary depending on the age of the birds and the general health status prior to the onset of infection.

## The Variant Strain:

In the 1980's, the United States saw an increase amount of downgrades in the slaughter plant. Further investigation revealed that birds are suffering from respiratory and other secondary infections i.e. Staphylococcus and *E. coli*. The underlying reason for this was found to be the non-responsiveness to the IBD, ND and IB vaccinations given. Intensive diagnostic testing revealed the presence of an IBD virus that is different from the classic Type 1 isolate that has been diagnosed for years.

The maternal derived antibodies (MDA) from the hens were not protecting the broilers from the infection. Immunosuppression from the virus is leading to susceptibility to all the other health challenges resulting to sub-standard performances and poor economic returns.

Some of the identified variants in Canada are (Ceren, on-going survey 2004):

- Delaware group (A, E)
- Group 6
- 586
- Arkansas 1568

even the head-associated lymphoid tissue (HALT). Microscopic lesions in the bursa are seen as inflammation with edema, necrosis and eventually lymphoid depletion and atrophy.

### 1. Occurrence and Clinical signs:

The variant strains are true antigenic variants in that they have altered or deleted antigenic sites vis-à-vis classic viruses. However, they are still 20-70% related. The disease usually does not show the typical clinical signs that are seen with the classic strains. However, infectivity is usually followed by field infections most notably respiratory related illnesses. Some of the reported health problems associated with IBD are: failure to respond to ND, IB and ILT vaccination, infectious bronchitis virus infection, reovirus infection (malabsorption, tenosynovitis, proventriculitis etc.) gangrenous dermatitis, inclusion body hepatitis, coccidiosis, Salmonella and *E. coli* infections, etc. Poor production performance is the most challenging effect of the virus. High feed conversion, poor uniformity, poor weight gain and average weight, high plant condemnation etc. have been associated with IBDV variants.

### 2. Gross and Microscopic Lesions:

The microscopic lesions of the bursa lack inflammation but only show as lymphoid necrosis. Re-population of lymphoid tissues is also delayed until 7 days after infection.

## The Very Virulent Strain (vvIBD):

At the same time that the US is dealing with variant IBD viruses, Europe, Africa and Asia start seeing acute cases of IBDV. These illnesses were being diagnosed in flocks at a later age and in farms that are on very good vaccination, biosecurity and management. The vvIBDV strains so far identified are 849 VB and DV86.

### 1. Occurrence and Clinical signs:

The clinical signs produced are similar to the classic virus but with higher morbidity and mortality (80% and 30% respectively). The illness spreads in a “very explosive but conserved manner” (van den Berg, 2000).

### 2. Gross and Microscopic Lesions:

The gross and microscopic lesions of the hypervirulent IBDV are similar to the classic virus but the acute phase is more severe and more generalized in the flock. It also tends to persist throughout the entire growing period of the birds. Hemorrhages are prominent in the pectoral and thigh muscles and in the bursa of Fabricius. The bursal lesions are very diagnostic. The thymus, spleen and bone marrow are affected more severely.

## What precipitated the evolution of the virus?

- Extreme vaccination pressure.
- 1. Use of cloned intermediate IBD vaccine that confers very narrow protection.
- 2. Vaccinating breeders with inactivated classic-type virus only resulting in chicks hatching with maternal antibodies of limited to the classic type.
- Short down time between grow-out.
- Improper cleaning and disinfection. Some growers “dry-clean” only by removing manure and blowing down dust.
- Increased bird population.

### **Q: When do I collect samples and what should I assemble together for submission to the laboratory?**

A. Routine or monitoring samples.

A good flock program should always include a monitoring plan in order to have a standard for comparing future laboratory results. Each operation differs because of unique management teams and protocols and therefore it is not always advisable to use other company’s data for comparison. “Base lining” is a good initial step for a sound management decision whenever it is needed.

## 1. Broilers

a. Know the level of maternal derived antibodies (MDA) by taking blood samples at day 1 in the hatchery or day 3 in the farm. Taking samples in the hatchery is easier than in the farm because the identity of the different breeder flocks can be traced with no trouble. Ten (10) vials of blood per flock done on a regular basis would be a good start. The MDA levels should be in excellent level during peak egg production. Generally, around 70% of the antibody from the hen is transferred to the progeny. These levels come down as the hens grow older. These MDA's would normally decay at the rate of 3-6 days (half-life) so that by 14-21 days of age, the antibody level would be gone regardless of the initial level (Lukert, 1991).

b. Some operations take another set of blood samples during harvest. The results aid them in monitoring for the presence of a disease challenge during the life of the flock. Normally, the presence of high titer readings would indicate a challenge.

## 3. Diagnostic Samples

The diagnosis of IBDV requires the collection of different samples in order to arrive at the correct conclusion. The following protocol is excellent for an IBDV survey:

1. Target flocks that are between 17 to 25 days of age. Some flocks may require more than one sampling (2, 3 and 4 weeks old) in order to get a good picture.
2. Gather 10 birds per flock, at random, and take blood samples for serology.
3. From the 10 birds, sacrifice 5 and collect the bursa of Fabricius.
4. The bursae can be evaluated grossly noting for inflammation or atrophy.
5. Each bursa is then cut in half where  $\frac{1}{2}$  goes to a 10% buffered formalin solution for histopathology and the other half is placed in a bag to be held in the freezer for molecular assaying (RT-PCR-RFLP), if needed.

## 2. Breeders:

Breeder birds are given live and inactivated IBD vaccines to protect them from the disease as well as to enable them to mount a good level of antibody for transfer to the progenies. Obtaining titer levels aids in assessing the efficiency of the vaccines, vaccination administration (crew), age of vaccination and general flock by flock response. Fifteen (15) blood samples should be collected at:

- 10 weeks live vaccine efficacy
- 25 weeks inactivated vaccine efficacy and pre-lay titers
- 35 weeks peak production titer levels
- 45 weeks mid-production titer levels
- 55 weeks to ensure that progenies are still sufficiently protected

## What are the different laboratory tests available for IBDV?

## A. Serology

1. Enzyme Linked Immunosorbent Assay (ELISA). The first kit that came to use was plainly called the IBD kit. This kit had an IBD classic virus (D78) as the antigen coated onto the microtiter plates. As such, serum samples with classic antibodies would react to the antigen with no problem. With the evolution of the virus however, it was soon realized that this kit was not detecting the field challenges with the variant strains. Thus a new kit was developed known as IBD-XR (Idexx). This kit can detect waning MDA and field challenges more accurately than the original kit. Titers with the XR kit are higher than the classic kit.
2. Agar Gel Precipitin (AGP) Test. This test measures group specific antigens (no serotype differences) and is not quantitative. In the UK, they have developed a quantitative test but this is not available in North America.
3. Virus Neutralization (VN). This test will detect the different serotypes and is the method of choice to discern antigenic variations among isolates. However, it is expensive and laborious.

## B. Virus isolation.

The bursa of Fabricius and spleen can be submitted to the laboratory for isolating the virus. Nine to 11 day old SPF embryos have been used with some success. Different routes of

## C. Histopathology.

Histologic lesions in the bursa of Fabricius are used to make initial diagnosis of IBD. Degeneration and necrosis of lymphocytes are good indicators of a challenge. In classic viruses, inflammatory reactions would also be visible.

The stage of the infection can sometimes be estimated by the pathologist based on the cellular changes and the re-population that eventually ensues. It must be remembered however that other insults can result to atrophy of the bursa.

## D. Molecular assay

Reverse Transcriptase-Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (RT-PCR-RFLP).

inoculation have yielded varying results. Variant strains can be differentiated from the classic strains by looking at the lesions in the embryos. The IBD virus has a broad antigenic diversity and some are fastidious thereby making this procedure challenging. Furthermore, some IBDV's may grow in embryos but can not be adapted in cell cultures.

PCR is a rapid procedure for synthesizing large amounts of DNA. A single strand of DNA can be amplified using PCR to  $1 \times 10^5$  to  $1 \times 10^6$  copies in several hours time. It became useful in laboratories in the late 1980's after the discovery of the enzyme that can synthesize DNA (*Taq* polymerase) and automation through a thermal cycler. With Reverse Transcriptase (RT) enzyme, RNA viruses (like IBDV) can also be subjected to the same test.

The bursa of Fabricius is taken and PCR is conducted. The nucleic acid is then further processed using an enzyme (RT) until the identification is fully determined by RFLP. This assay enabled the placement of viruses into genetically-related groups called molecular groups (Jackwood). Currently, there are 6 molecular groupings composed of classic and variant strains. New patterns are also discovered as more samples are analyzed. These new strains are identified using gene sequencing or real time RT-PCR.

It is very important that IBD is monitored and proper diagnosis is done when needed. The disease can be in the farm sub-clinically affecting production performance without outright morbidity and mortality.

## **Control and Prevention:**

The approach to IBDV control and prevention involve knowing what you have in your farm and your region. A good biosecurity, cleaning and disinfection, sufficient down time and good chick source are important to ensure that the birds would be competent to face any disease. Vaccines that cover both classic and variant strains are available. Suggested vaccination program would include:

1. Breeders – Use both classic and variant strains.
  - a. Live attenuated vaccines, 2- 3 doses depending on the challenge.
  - b. Inactivated vaccines – 2 doses
2. Broilers – Currently, there is approximately 50% broiler flocks in Canada that are

vaccinated. Some researchers have shown that birds given day old vaccination performed better.

In countries that have the vvIBDV, prevention has become quite difficult because of the circumstances of its occurrence in vaccinated and well-managed flocks. Currently, control program calls for a live (including potentiated) and killed vaccination in breeders and an attenuated intermediate vaccine in broilers. The selection of the vaccines is very vital to ensure that quality antigens are provided.

Scientists are now looking at new vaccines (DNA, recombinant etc.) as well as the role of cell mediated immunity.

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