

from the president...

Our technical group's efforts in manufacturing and research are achieving excellent results. We have tripled our production capacity of Marek's vaccine and other live vaccines since we began the company, and our success rate for serial release has shown incredible improvement.

We cut the ribbon on the \$4.5 million expansion of our new production facilities in June. Our objective is to increase our inactivated capacity and to lower our costs, thereby increasing our competitiveness. Additionally, the facility's GMP status will allow shipment of product into the EU, another step in the expansion of our opportunities.

To streamline and improve our product distribution, we consolidated United States and Canadian distribution into our Gainesville, Ga., site. With multiple cold storage facilities, computerized links to both manufacturing sites and experienced personnel, we can provide quick, efficient



*Dave Zacek,
President of
Lohmann
Animal
Health
International,
Gainesville,
Georgia,
USA*

delivery of vaccine to our customers in this important market area. Both MBL and Vineland vaccines can be sourced from this location by calling toll free 800-655-1342 beginning July 1, 2001. Inventories, sent via refrigerated trucks to Gainesville, will be on hand to supply immediate delivery requests.

Now in the final stages of development, our new 30,000-square foot commercial, packaging and distribution center in Vineland, New Jersey, will be completed and operating August 1, 2001. This new facility is geared to satisfy the

specific needs of our international customers. On-line labeling will allow for custom label requirements to be met near the time of order processing.

To better serve our customers and support our distributor partners, we divided sales management of the Vineland, Maine Biological Laboratories and Lohmann Animal Health brands in July. Under this structure, LAHI, based in the U.S., serves North, Central and South America, and LAH, based in Germany, serves the rest of the world. Each manufacturing center will continue to supply technical and registration information about their specific products, but day-to-day sales and marketing will correspond with geographical lines.

We are proud of these efforts, showing what a dedicated group of people can accomplish with the proper resources. Our success translates into excellent supply and service for our global customer base, encompassing poultry professionals in more than 56 countries.

avian insight

A LOHMANN ANIMAL HEALTH INTERNATIONAL NEWS BRIEF

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Infectious Bursal Disease Virus: A Continually Moving Target

*Joseph J. Giambrone
Professor of Poultry Science
Auburn University, Auburn, AL 36849-5416
jgiambro@acesag.auburn.edu
<http://www.auburn.edu/~giambjj/>*

Introduction

Infectious bursal disease (IBD), often referred to as bursal or Gumboro disease, has been a constant thorn in the side of the commercial chicken industry since its discovery in Gumboro, Delaware, in the late 1950s. In the 1960s and '70s, this highly contagious viral disease mainly appeared in the clinical form, affecting chickens between two and four weeks of age. Affected birds (about 30% of the flock) were pale, ruffled-up, lethargic, had an unsteady gait, and white or reddish diarrhea. Mortality was moderate (20% or less) and weight gain and feed efficiency were poor.

Hot Vaccines

The virus was resistant to most disinfectants at the time and, therefore, was controlled through the use of fairly virulent live vaccines consisting of ground homogenates from the bursa of Fabricius of chickens infected with field isolates. The bursa is the tiny lymphoid organ in

the hindgut of all birds, and is the target organ for replication of the virus, which causes the disease. These homogenates were given to 1- to 2-week-old chickens by drinking water. This would result in a mild form of the disease with about 5% mortality and 10% morbidity. Developed in the southern United States, this form of controlled exposure, otherwise known as "fighting fire with fire," would again come to the forefront 25 years later in Europe for the control of very virulent IBD virus (V). Nevertheless, these fairly virulent vaccines given to very young chickens significantly reduced the clinical form of disease seen prior to their use in the field.

Milder Vaccines

In the 1980s came the milder, embryo and cell culture derived vaccines. These products were much safer than previous vaccines and were widely used throughout the world. Although these viruses adequately controlled the clinical form of IBDV, a new, previously unrecognized form appeared. The virus was found to produce atrophy of the bursa of Fabricius and immunosuppression, when chickens were infected with the virus during the first few weeks of age. This form did not cause clinical disease, and, therefore, was known as the subclinical immunosuppressive form. This form decreased the immune response of infected chickens to subsequent vaccination and increased their susceptibility to pathogenic organisms in the field. Affected flocks would have a higher incidence of respiratory, enteric, and integumentary diseases. These flocks often had much higher pro-

cessing plant condemnations resulting in considerable economic losses. The control of this subclinical immunosuppressive form required the use of a combination of live and newly developed inactivated vaccines in the breeder pullet flocks. These hyperimmunized hens then passed high and uniform amounts of maternal antibody to their progeny. This passive immunity would protect the chicks for the first two to three weeks of age.

Antigenic Variants

With the widespread use of live and killed vaccines in the parent flocks, the spread of highly concentrated and intensive chicken populations, and the use of rearing flocks on built-up litter, a major change occurred in the IBDV's molecular structure.

New antigenic variants first appeared in Delmarva Peninsula in the late 1980s and then spread throughout the world in the 1990s. These viruses underwent a change in the structure of the VP2 gene, which helped them survive in the poultry industry, where vaccination was common. This gene codes for the major viral capsid protein, which induces neutralizing antibody in the infected chicken. A small segment of this gene, less than 120 nucleic acid base pairs in length, is where the important variation occurred. Only one base alteration in the right area caused a change in a critical amino acid. This change altered the binding site of the virus for the antibody, epitope, which bestowed on the virus the ability to escape neutralization by antibody. This genetic site in the VP2 is known as the hypervariable region. These variant

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viruses were first detected using cross virus neutralization tests in cell culture.

Later panels of monoclonal antibodies were developed that could differentiate antigenic standard (or classic) from variant viruses (Table 1). These first antigenic variants, commonly referred to as escape mutants, were also pathogenic variants in that they did not cause mortality or the inflammatory phase, which resulted in a large edematous bursa. These isolates, instead, caused a rapid atrophy of the bursa and severe immunosuppression, even in chickens with significant amounts of maternal immunity against previous efficacious classic IBDV vaccines.

A common misconception about these new viruses is that they do not cause morbidity. In numerous studies done at Auburn University over the past 10 years with a variety of these antigenic variants isolated from regions all over the United States, we have found that most of these isolates caused diarrhea and weight loss. Therefore, poultry producers suffering from any adverse weight gains during the first weeks of age, should examine their IBDV vaccination program. Use of serologic monitoring of breeder flocks is helpful, but the best test is live challenge of progeny at two weeks of age with both variant

and standard IBDVs. Birds are then sacrificed at one week post-challenge and observed for the percentage of the chickens with normal, non-atrophied bursa.

Seven years and some 50 trials of this nature have been done at Auburn. Our results have shown that producers are doing an excellent job of controlling the antigenic standard IBDV, but protection against variant IBDVs could still be improved (Table 2). New live and killed vaccines containing standard and antigenic variants, and killed vaccines produced from the bursa of Fabricius or bursal cell culture lines, are helpful for improved IBDV control. Virus propagated from bursal-derived tissue or cells has a structure more similar to the field virus and, therefore, will induce a higher percentage of neutralizing antibody than vaccine virus grown in standard chicken embryo fibroblast cell culture. Also, the best protection requires at least one live intermediate and one intermediate plus vaccine, along with two killed vaccines in the pullet flocks. Though not widely practiced, use of a third killed product during the pullet stage, or better yet during lay, is also helpful in boosting immunity in older hens. Our data shows that, with hen age, immunity declines faster against the antigenic variants than the standard viruses.

TABLE 1

AC ELISA CHARACTERIZATION OF IBDV ISOLATES						
Mab	Ark	Ga	Miss	Vine	APHIS	
R63	+	+	+	+	+	
B69	-	-	-	-	+	
B29	+	+	+	+	+	
3-1	+	-	+	-	+	
39A	+	+	+	-	+	
9-6	+	+	+	+/-	+	
44-10	+	-	+	-	+	
17-82	+	-	+	-	+	
33-10	+	+	+	-	+	

Table 1. Results of monoclonal antibodies and an Antigen Capture ELISA test used to differentiate IBDVs. Antibodies are listed under the column marked with Mab and the five viral isolates (Ark, Ga, Miss, Vine, and APHIS) are listed at the top of the other columns. The first four viruses are all antigenic variants, whereas the APHIS is the standard challenge virus. The APHIS is the only virus that reacts with the B69 Mab. The reactions are either positive (+) or negative (-) with one in between (+/-).

Pathogenic Variants

In the 1990s another major change in the IBDV occurred. The virus greatly increased its virulence resulting in as much as 90% mortality and 100% morbidity in susceptible white leghorn strains. These isolates were named very virulent viruses (vvIBDV). Originally isolated in Europe, these viruses quickly spread to Asia and Latin America. As of 2001, they have not been reported in North America. These viruses also have caused changes in the hypervariable region of the VP2 gene; however, researchers are not sure if these genetic changes directly result in these pathogenic changes or are just coincidental markers that can be used to distinguish them from other less pathogenic viruses. These viruses were initially controlled with less attenuated “intermediate plus” vaccines in the drinking water, similar to those used in the 1960s in the U.S. Years of using these vaccines can cause immunosuppression, so poultry producers have successfully switched to more attenuated intermediate products for good control of very virulent IBDVs without the resulting immunosuppression. Usually two vaccinations are given during the first 18 days of age — one in the hatchery and the other in the field — by drinking water or coarse spray.

IN OVO Vaccination

Another successful method for administration of IBDV vaccines has been the *in ovo* route. This route was developed for Marek’s disease (MD) vaccination in the U.S., and has caught on rapidly in much of the modern world in response to rising labor costs. Using this method, two people can vaccinate as many as 60,000 embryos per hour. MD vaccines are often mixed or bought in the same bottle with IBD vaccines for delivery to 18-day-old broiler embryos. Work at Auburn University has shown that most intermediate vaccines are safe and efficacious against both antigenic standard and variant viruses till the birds are five weeks of age, when given by *in ovo*.

TABLE 2

IBDV PROGENY CHALLENGE RESULTS				
Flock #	STD	VarE	Avg	VN
1	100	68	84	128
2	100	18	59	10
3	100	30	68	60
4	30	5	11	10

Table 2. Results show % protection scores and virus neutralization titers for each of four broiler flocks that were challenged at two weeks of age with the standard (STD) APHIS or Var E IBDV. Flocks 1 and 3 were taken from the same breeder flock and flocks 2 and 4 were from different flocks. Serum was taken at the time of challenge using a standard virus as indicator in a cell culture system. Flocks 1 and 3 are well protected (high average % protection and good VN antibody titers), whereas flocks 2 and 4 were not. These two breeder flocks should receive another killed IBDV vaccine booster dose.

A new vaccine (BursaPlex) combines an intermediate IBDV vaccine complexed with serum against the virus. The antibody in this immune complex vaccine delays the replication of the virus so that it will not induce immunosuppression. This vaccine has shown efficacy in the field against very virulent IBDVs in Europe. Our work at Auburn University has also shown that this complex product will induce a cell-mediated, but not antibody response, resulting in immunity to the virus.

More Genetic Variations

The past decade has also seen further shifts in the molecular structure of the VP2 gene. Using monoclonal antibodies in the antigen capture ELISA test, as well as the polymerase chain reaction (PCR) and restriction fragment polymorphism test (RFLP) (Table 3), numerous researchers have shown that field viruses continue to mutate, resulting in escape mutants. These antigenic variants are now appearing all over the world, causing vaccine failures. This necessitates the development of new killed vaccines containing homologous field strains to control these viruses. The author sees no harm that can evolve from the use of new killed vaccines containing homologous field isolates. However, widespread use of new live variant viral vaccines should be done cautiously, because these viruses may introduce new variants in areas where they did not previously exist. Laboratory conformation of the antigenic and pathogenic nature of field

viruses is essential to produce safe and adequate control of the virus.

Infectious Proventriculitis

The latest IBDV variants to evolve are viruses that can cause or contribute to infectious proventriculitis. This enteric syndrome is characterized by reduced weight gain, processing plant stoppages, and immunosuppression. Affected birds can have diarrhea, atrophy of the bursa and sometimes the thymus, and an enlarged flabby proventriculus. The proventriculus may have enlarged glands and edema, and can burst open during processing, resulting in condemnation from contamination of digested material on the edible carcass.

This disease was first associated with IBDV by the late Dr. Kirk Skeeles at the University of Arkansas about four years ago. We have been working for the past two years with one of his isolates and several of our own from North Alabama broiler flocks. These isolates came to us by way of researchers (Drs. Tami Kelly and Fred Hoerr) from the Alabama State

TABLE 3

RESTRICTION ENZYME SITES WITHIN THE HYPERVARIABLE REGION OF THE IBDV VP2 GENE					
Rev	Ark	Ga	Miss	Vine	APHIS
EcoR II	1	1	2	1	2
Hae III	3	3	3	3	4
Stu I	0	0	0	0	1
Sty I	1	1	1	0	2

Table 3. The PCR-RFLP test can be used to differentiate the four variants from the standard APHIS viruses. The APHIS is the only virus that is cut with the Stu 1 restriction enzyme. The Ark and Ga isolates revealed the same pattern (same number of sites) in the agarose gel and cannot be differentiated using this test.

Veterinary Diagnostic Laboratory System. They can reproduce a similar disease in SPF leghorns or broilers with either bursal or proventricular homogenates, isolated from infected commercial chickens. These homogenates contain antigenic variant IBDVs. We have also shown that live IBDV vaccines can significantly reduce this disease in laboratory trials. We are presently incorporating these isolates in our live IBDV progeny chick challenge model to see what effect passive immunity has on the incidence and severity of this disease.

Again, it is important to re-examine your IBDV vaccination program if you are seeing proventriculitis in the processing plant or have suboptimal weight gain and feed conversion in broiler flocks.

Summary

IBDV is a pathogen that is alive and well in this millennium. This RNA virus has the uncanny ability to change its genetic structure in order to survive a continual barrage of new vaccines. We will no doubt be discussing this disease through the end of this decade and possibly the next. New recombinant DNA vaccines incorporated into multivalent *in ovo* vaccines are now being tested in the field. Transgenic plants incorporating the VP2 gene of standard and antigenic IBDVs are being developed in our laboratory. These plants will be processed into future edible vaccines and placed in the diet of chickens as part of field vaccination procedures. The future of IBDV control, will indeed, be interesting.