MONITORING THE IMMUNE STATUS OF BROILER BREEDERS AGAINST INFECTIOUS BURSAL DISEASE VIRUS USING PROGENY CHALLENGE AND SEROLOGIC DATA

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SUMMARY

Infectious bursal disease virus (IBDV)-induced immunosuppression causes sub-optimum performance in broilers. Integrators use enzyme-linked immunosorbent assay (ELISA) as an indication of breeder vaccine success. The IBDV challenge of progeny correlates more closely to performance than does ELISA. Four IBDV serologic and challenge studies were undertaken using progeny from integrators to determine the efficacy of vaccination programs. All breeders received two live and two inactivated vaccines. For each experiment, day-old progeny were taken from six breeder flocks. At 2 wk, 15 progeny per flock were challenged with the serologic standard (STD) virus, 15 per flock with variant E, and 15 per flock with one of four variants (GA, ARK-1, ARK-2, and MISS). Percent protection for the STD were close to the MISS and ARK-1 isolates, whereas percent protection for the ARK-2 and GA isolates was similar to variant E. The new ELISA was improved over previous products and correlated with resistance to challenge against the STD, MISS, and ARK-1, but not with the other isolates. Therefore, producers must improve their vaccination programs or risk continued problems due to antigenic variants.

Key words: Challenge, IBDV, immune suppression, monitoring, progeny

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DESCRIPTION OF PROBLEM

Infectious bursal disease virus (IBDV)-induced immunosuppression continues to be a problem in commercial broilers, resulting in increased condemnations, above average mortality, and suboptimal feed conversion, and weight gains [1]. Antigenic variants of IBDV and/or poor broiler breeder pullet vaccination techniques plague the U.S. industry, resulting in increased susceptibility to subclinical IBDV immunosuppression [2]. Recent studies [3, 4] have shown that IBDV variants predominate in U.S. broiler-producing areas. Variant IBDV have been recently isolated from Georgia [5], Mississippi [1], and Arkansas [6]. Maternal immunity derived from breeder vaccination represents the first line of defense against early IBDV infections. Pullet vaccination success is based on serologic profiling of the hens during egg production. However, the two most commonly used commercial enzyme-linked immunosorbent assay (ELISA) kits use a serologic standard antigen as the indicator. Antibody titers using this kit do not correlate well with broiler flock performance [7]. Another means of determining the susceptibility of progeny to IBDV infections is to measure the size of the bursae during the grow out. However, there are numerous infectious and noninfectious agents which can alter the size of the bursae [1].

One researcher [7] has developed an IBDV progeny challenge model for determining the success of breeder hen vaccination programs. Using broiler progeny from the Delmarva peninsula, he has determined that there is a direct correlation between resistance to the Delaware variant E virus challenge at 2 wk of age and performance in the field. The bursa weight:body weight ratio was correlated with resistance to IBDV infection. The greater the ratio the more resistant the bird was to challenge. This very important field study initiated our current experiments.

The purpose of these experiments was to implement this IBDV challenge program [7] at Auburn University and to determine the efficacy of broiler breeder vaccination programs in commercial flocks around the southeastern U.S. using newly isolated antigenic variants from the southeastern U.S. and a new ELISA kit containing both STD and variant IBDV.

Challenge results were determined using both bursae size and microscopic pathology. However, we did not have access to the broiler performance data as did the prior study. In order to avoid promoting one product over another, we did not intend to determine which vaccine or program is the best, but rather to determine whether current vaccines and programs commonly used in the industry would protect against newly isolated variants of IBDV and if the new ELISA system containing both STD and bursal-derived variant IBDV antigens would correlate with resistance to challenge at 2 wk of age with the variant IBDV.

MATERIALS AND METHODS

Chickens were challenged by eye and nasal instillation with $10^{7.5}$ID$_{50}$ of the serologic standard challenge virus from the Animal Plant and Health Inspection Service (APHIS) in Ames, IA or the Delaware antigenic variant E virus [8].

Broilers were from commercial breeder flocks from Arkansas, Alabama, Georgia, Mississippi, and North Carolina. Breeder flocks ranged in age from 30 to 50 wk when the eggs were taken and hatched. All breeders were vaccinated as pullets with two live and two inactivated IBDV vaccines. The vaccines were from various commercial U.S. manufacturers and the inactivated vaccines contained both serologically standard and variant E IBDV. The first live vaccine contained an intermediate STD IBDV and was given during the first 2 wk of age. The second live vaccine contained an intermediate plus vaccine (less attenuated and more virulent than the first vaccine) and was given during 6 to 10 wk of age. The first inactivated vaccine was given between 10 and 14 wk of age and the second between 18 and 20 wk of age. All inactivated vaccines contained at least 50% bursal-derived antigen.

Vaccines containing bursal-derived antigens have been shown to be more protective than antigens from cell culture because IBDV propagated in cell culture results in many noncomplete viral particles which may induce antibodies which are non-neutralizing. Since the authors were not present during the pullet vaccinations, we cannot comment as to the effectiveness of vaccine administration. The commercial vaccine names for each breeder flock were known, but were not revealed to...
avoid commercialism. The objective of these experiments was not to determine which vaccine or program was the best, but rather to determine whether current vaccines and programs commonly used in the industry would protect against newly isolated variant IBDV, and if the new ELISA system containing both STD and variant IBDV antigens would correlate with resistance to challenge at 2 wk of age with the variant IBDV.

All broilers were fed a commercial broiler starter and given feed and water ad libitum. All were held in modified Horsfall-Bauer isolation units maintained with filtered air under negative pressure.

Ten birds per flock were bled at 2 wk of age and sera analyzed for antibody against a standard IBDV antigen using a commercial ELISA kit. The kit was from Kirkagaard and Perry Lab, Inc. (KPL) of Gaithersburg, MD and contained an STD (D78) and variant E antigen produced from bursal-derived material. This fact is important since most inactivated vaccines contain variant E produced in the bursa.

A total of four challenge studies were done during an 8-month period. Each represented chickens from a separate integrator. Progeny from six breeder flocks were tested during each experiment. Fifty-five chicks were obtained from each broiler flock for a total of 330 birds per experiment. At 2 wk of age, 15 chicks from each flock were challenged with one of the three IBDV’s (Groups 1–3) and 10 chicks (Group 4) were not challenged. In each of the four experiments, Group 1 chicks were challenged with the antigenic STD IBDV ARK-1 isolate, and Group 2 with the antigenic Delaware E variant [8]. In Experiment 1, Group 3 [3] was challenged with the antigenic variant GA isolate [5]. In Experiment 2, Group 3 received the variant MISS [1] isolate. In Experiment 3, Group 3 received the variant ARK-1 [6], and in Experiment 4, Group 3 received the variant ARK-2 IBDV. The ARK-2 IBDV was isolated in 1997 from the Northwest Arkansas and determined to be variant using molecular techniques [3]. The 10 control birds per flock were also bled at this time and sera measured for antibodies against IBDV using the ELISA. The four groups were kept in separate isolation units.

At 7 days after challenge, all birds were killed, weighed, and their bursae extracted and weighed. Bursae weight:body weight ratios were determined for each of the challenge groups with chicks from each of the six breeder flocks. All bursae were placed in formalin and processed for microscopic observations using Hematoxylin and Eosin staining. Bursae were scored from 0 to 4 based on increasing severity of lesions [9].

Bursae weight:body weight ratios were analyzed using the Statistical Analysis System (SAS) software (version 9.1). The percent protection was determined based on the percentage of birds within a flock that were protected. Flocks with a percent protection score above 30% were considered adequately protected. This figure was derived from a previously published work [7]. In that study, chicks from flocks with percent protection scores below 30% had poor field performance. In the present experiments, we did not have access to broiler flock performance.

RESULTS AND DISCUSSION

Table 1 presents IBDV serologic and challenge data, based on bursa:body weight data (% protection scores) for Experiment 1. Data showed that all flocks, except Flock 6, were well protected against the STD challenge. Results for the IBDV E and GA variants were similar. All flocks, except Flocks 5 and 6, were adequately protected. In Experiment 2, results for the STD and MISS isolates were similar (Table 2). All flocks, except Flock 1, were adequately protected against these two isolates. In contrast, only Flock 3 was adequately protected against variant E. In Experiment 3, challenge results for the STD and ARK-1 isolates were similar (Table 3). All flocks were adequately protected against these isolates. In contrast, only Flock 2 was adequately protected against variant E. In Table 4, data for Experiment 4 showed that only Flocks 1, 2, and 3 were adequately protected against the STD isolate. Results for the variant E and ARK-2 isolates were similar. Only Flock 2 was adequately protected against these isolates.

In the interest of brevity, microscopic lesion scores were not listed in table form. Results correlated with data calculated from the bursa weight:body weight ratios. Results
were also variable for individual flocks and IBDV isolates. They ranged from a low of 0 to a high of 4. Flocks with an average lesion score of 2 or less were generally adequately protected against IBDV as measured by the bursal weight:body weight ratios.

Individual geometric mean ELISA antibody titers (GMT) at 2 wk of age for a broiler flock ranged from a low of 14 to a high of 16,112. The correlation of variation (CV) was low (less than 10%) for all flocks tested. Flocks with GMTs of 1,500 or more were always adequately protected against the STD challenge. However, correlation between ELISA analysis and resistance to the variant E isolate was not consistent. Flocks with antibody titers as high

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**TABLE 1. IBVD challenge results of progeny (Experiment 1)**

<table>
<thead>
<tr>
<th>FLOCK #</th>
<th>STD²</th>
<th>VARIANT E</th>
<th>GA</th>
<th>ELISA³</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>70</td>
<td>66</td>
<td>58</td>
<td>5,391</td>
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<tr>
<td>2</td>
<td>92</td>
<td>92</td>
<td>92</td>
<td>11,687</td>
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<tr>
<td>3</td>
<td>82</td>
<td>90</td>
<td>70</td>
<td>3,633</td>
</tr>
<tr>
<td>4</td>
<td>54</td>
<td>86</td>
<td>30</td>
<td>4,657</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>8³</td>
<td>25³</td>
<td>4,406</td>
</tr>
<tr>
<td>6</td>
<td>23³</td>
<td>20³</td>
<td>18³</td>
<td>6,513</td>
</tr>
</tbody>
</table>

A³STD = Serological standard APHIS IBDV.
B³Geometric mean enzyme-linked immunosorbent assay.
C³Statistically different from other numbers within the same column.

**TABLE 2. IBVD challenge results of progeny (Experiment 2)**

<table>
<thead>
<tr>
<th>FLOCK #</th>
<th>STD²</th>
<th>VARIANT E</th>
<th>MISS</th>
<th>ELISA³</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14³</td>
<td>27³</td>
<td>24³</td>
<td>56</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>6³</td>
<td>87</td>
<td>1,502</td>
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<td>3</td>
<td>65</td>
<td>54</td>
<td>75</td>
<td>3,514</td>
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<tr>
<td>4</td>
<td>37</td>
<td>0³</td>
<td>94</td>
<td>4,235</td>
</tr>
<tr>
<td>5</td>
<td>53</td>
<td>23³</td>
<td>100</td>
<td>3,628</td>
</tr>
<tr>
<td>6</td>
<td>60</td>
<td>27³</td>
<td>60</td>
<td>6,152</td>
</tr>
</tbody>
</table>

A³STD = Serological standard APHIS IBDV.
B³Geometric mean enzyme-linked immunosorbent assay.
C³Statistically different from other numbers within the same column.

**TABLE 3. IBVD challenge results of progeny (Experiment 3)**

<table>
<thead>
<tr>
<th>FLOCK #</th>
<th>STD²</th>
<th>VARIANT E</th>
<th>ARK-1</th>
<th>ELISA³</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>87</td>
<td>14³</td>
<td>100</td>
<td>14,860</td>
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<td>2</td>
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<td>4</td>
<td>74</td>
<td>6³</td>
<td>87</td>
<td>12,775</td>
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<td>5</td>
<td>94</td>
<td>0³</td>
<td>94</td>
<td>11,561</td>
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<tr>
<td>6</td>
<td>80</td>
<td>6³</td>
<td>94</td>
<td>16,112</td>
</tr>
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</table>

A³STD = Serological standard APHIS IBDV.
B³Geometric mean enzyme-linked immunosorbent assay.
C³Statistically different from other numbers within the same column.
as 16,112 were poorly protected against this isolate. Therefore, producers with high titers using the new ELISA system may mistakenly believe they their broiler progeny are adequately protected against antigenic variants. Flocks with titers, below 1,500 were always poorly protected against these isolates. Investigators from a prior study [1] did not have access to this new ELISA system, so we cannot compare their ELISA results to ours.

IBDV-induced immunosuppression continues to cause significant economic losses in the U.S. broiler industry despite improved vaccine programs. All commercial broiler breeder pullets in the U.S. are now receiving combinations of live and inactivated vaccines containing both serologic standard and variant viruses. Recent studies have shown that the incidence of variant IBDV is increasing throughout the U.S. [3, 4] and that resistance to IBDV variant challenge at 2 wk of age correlates with performance in the Delmarva area more so than does serologic profiling of breeders using commercial ELISA kits [7]. Previous results showed that resistance to the variant E virus was significantly less than to the standard virus. That study had access to broiler performance data not available in the present study. Researchers showed that flocks that had less than 30% protection against STD and variant IBDV at 2 wk of age performed poorly in the field.

Data presented in the tables in this study does not reveal the age of the breeders when the eggs were taken for the progeny challenge studies. However, with hen age the protection against the variant challenge waned, but was relatively constant for the STD challenge. Breeder flocks older than 40 wk produced progeny that had lower ELISA titers and were more susceptible to challenge against the variants than were progeny from younger breeder flocks.

In the present experiments, progeny were taken from states which represent nearly 60% of the U.S. broiler production. Our data confirmed previous results [7], which showed that producers from Delmarva were doing a good job of immunizing their flocks against the standard virus, but significantly less so against the variant E. Our data also agreed with previous work [7], which showed a lack of correlation between ELISA titers and resistance to the variant E virus as well as the newly isolated ARK-2 and GA variants at 2 wk of age. However, since we did not have access to the broiler performance data, we cannot make assumptions as to the correlation of ELISA titer and percent protection scores with broiler flock performance.

Viruses have many antigenic sites which are capable of eliciting an antibody response in the host. Some sites elicit neutralizing and some non-neutralizing antibodies. The ELISA does not measure the neutralizing IBDV antibody, and therefore there is not always good correlation between ELISA and resistance to IBDV infection. In addition, resistance to IBDV infection may also rely on cell-mediated immunity. However, this new ELISA from KPL was an improvement over previous kits since titers of 2-wk-old birds were higher and CV's were lower than data generated with older kits in similar studies. Also, there was good correlation between the ELISA titer and protection against the STD, MISS, and ARK-1 viruses.

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TABLE 4. IBVD challenge results of progeny (Experiment 4)

<table>
<thead>
<tr>
<th>FLOCK #</th>
<th>STD&lt;sup&gt;a&lt;/sup&gt;</th>
<th>VARIANT E</th>
<th>ARK-2</th>
<th>ELISA&lt;sup&gt;b&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td>1</td>
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<td>0</td>
<td>3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8,156</td>
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<tr>
<td>2</td>
<td>86</td>
<td>54</td>
<td>32</td>
<td>9,040</td>
</tr>
<tr>
<td>3</td>
<td>86</td>
<td>24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8,720</td>
</tr>
<tr>
<td>4</td>
<td>24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15</td>
</tr>
<tr>
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<td>14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>6</td>
<td>24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14</td>
</tr>
</tbody>
</table>

<sup>a</sup>STD = Serological standard APHIS IBDV.
<sup>b</sup>Geometric mean enzyme-linked immunosorbent assay.
<sup>a</sup>Statistically different from other numbers within the same column.
Since IBDV infections continue to occur in the U.S. and new antigenic variant viruses continue to evolve such as recently occurred in Georgia [5], Mississippi [1], and Arkansas [6], continual monitoring of IBDV pullet vaccination programs using a number of serologically different IBDV’s seems warranted. In addition, the MISS and ARK-1 variants are both antigenically and pathologically (cause inflammation of the bursae) distinct from the Delaware E, GA, and ARK-2 variants.

Further studies would also be warranted to correlate resistance to challenge infection with the presence of virus-neutralizing antibodies and/or cell-mediated immune response against the homologous antigenic variant virus. Other future studies could also examine whether the use of new live variant vaccines, inactivated vaccines containing a higher percentage of bursal derived material in pullet flocks, and/or a mid lay boost with an inactivated vaccine would provide better protection against variant IBDV’s.

CONCLUSIONS AND APPLICATIONS

1. Producers continue to do a good job in vaccinating broiler breeder pullets to prevent infection against serologic standard IBDV in the progeny, but results are variable against the newly evolving variant IBDV’s.
2. The vaccines and programs tested in this study were effective in controlling the five antigenic variant IBDV’s we examined.
3. The new ELISA test containing both STD- and bursal-derived variant E antigen correlates well with resistance to protection against the STD IBDV and with some but not all antigenic variants.

REFERENCES AND NOTES


ACKNOWLEDGEMENTS

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