

**THE EMERGENCE OF NEW-TYPE VARIANT IBV VIRUSES IN
THE UNITED STATES AND HOW TODAY'S BURSAL
DERIVED VACCINES PROTECT AGAINST THEM**

Kalen Cookson, DVM, MAM
Fort Dodge Animal Health, Overland Park, Kansas.

INTRODUCTION

Infectious bursal disease virus (IBDV) infections before 2 weeks of age can cause profound and long-lasting immune suppression in chickens. For the past 15 years, hyperimmunization of broiler breeders using two separate injections of bursal tissue origin (BTO) inactivated vaccines has provided the best levels of progeny protection. However, IBDV is notorious for its ability to antigenically change over time in response to a constant pressure—namely, all conventional BTO vaccines contain Delaware E as their primary immunogen. As a result, there has been an emergence of New-type IBDVs that are genetically distinct from the Delaware viruses (3,4,5,6).

According to a recent U.S. field survey of almost 50 IBD viruses from problem farms, phylogenetic analysis showed that about half of the samples fell into a separate lineage (called Branch-1) from the Delaware family (6). Some of these New-type IBD viruses, such as T1 and AL-2, can overcome higher levels of conventional BTO derived antibodies, resulting in lower protection rates in 7-14 day broiler challenge models (4,10).

In the progeny challenge studies summarized here, the ability of four New-type variant viruses to infect broilers at 2 weeks of age was compared to Del-E. Also compared was protection conferred by various conventional BTO programs vs. programs where a BTO vaccine containing a Branch-1 (Br-1) IBDV was substituted in one of the two vaccinations.

MATERIALS AND METHODS

Broilers came from prime-age breeders located in four distinct geographic regions—Alabama, Texas, Arkansas and Delaware. A total of five different conventional vaccines were represented in the following combinations: Alabama (D/C), Texas (E/B), Arkansas (B/B) and Delaware (A/A). At each site, comparison flocks received a BTO vaccine containing a Br-1 in the first shot at Alabama and Arkansas and in the second shot at Texas and Delaware. Vaccines A and B are #1 and #2 in the U.S. in sales and the Br-1 vaccine was substituted for them in three of the four trials. All broilers were vaccinated *in ovo* with Marek's and IBD vaccines.

Broilers from Alabama, Texas and Arkansas were housed in Horsfal isolator units at Auburn University at 20 per group. At 14 days of age, groups were challenge by eye and nose drop inoculation with 3.5 log₁₀ of one of the following: Del-E, AL-2 or Ark-6. Ark-6 is a Jackwood RFLP Group 6 virus that was isolated from a farm with a history of various disease problems, poor performance and lymphocytic proventriculitis.

Broilers from Delaware were housed in colony houses at the Lasher Laboratory and challenged with Delaware-E and one of three AL viruses: AL-2, AL-10 and AL-19. AL-2 and AL-10 are New-type variant IBDVs with very unique antigenic properties (9).

At 21 days of age birds were sacrificed and bursas and body weights were measured. Gross bursal protection was calculated based on the number of challenged birds whose bursa to body weight ratio (B:BW) was higher than the cut-off standard (mean B:BW minus 2 standard deviations) of the respective control group.

RESULTS

In all four vaccine trials, programs containing one shot of the Br-1 containing vaccine had equal or better B:BW protection levels against every IBDV tested. Broilers challenged at Auburn (Table 1) were from sister flocks raised on the same farm, while the results at Delaware (Table 2) are an average of 10 similar-aged flocks on each program.

**Table 1. Auburn Univ. study (Giambrone et al),
% bursal protection**

| Trial site | Killed prog. | Del-E gross/histo | | AL-2 gross/hist. | | Ark-6 gross/hist. | |
|------------|--------------|-------------------|-----|------------------|-----|-------------------|-----|
| | | | | | | | |
| Alabama | D/C | 58* | 53* | 70 | 45* | 47 | 26* |
| | Br-1/C | 90 | 86 | 65 | 70 | 60 | 60 |
| Texas | E/B | 90 | 79 | 45 | 21* | 50* | 45* |
| | E/Br-1 | 85 | 85 | 80 | 60 | 90 | 95 |
| Arkansas | B/B | 50 | 40 | 61 | 0* | 60 | 50* |
| | Br-1/B | 65 | 50 | 55 | 50 | 68 | 80 |

* Flocks with significantly lower % protection within a trial site (Tukey's exact test, P<0.05).

**Table 2. Lasher Lab study (Salem et al),
B:BW % protection***

| Trial site | Killed program | Del-E | AL-2 | AL-10 | AL-19 |
|------------|----------------|-------|------|-------|-------|
| Delaware | A/A | 63 | 35 | 20** | 42 |
| | A/Br-1 | 76 | 41 | 32 | 59 |

* These 20 flocks were not analyzed for protection based on histological damage (imaging analysis).

** Average flock protection is considered poor.

DISCUSSION

The use of IBD vaccines mixed with Marek's will tend to enhance the protection levels in commercial broilers. This has recently been demonstrated when given either at day of age (1,7) or *in ovo* (2,8). Since flocks submitted to Auburn all received *in ovo* IBD vaccine, protection rates below 30% would be considered especially susceptible to challenge. Based on B:BW analysis, all these flocks were reasonably protected, although each conventional flock fell below this minimum standard based on imaging analysis against either AL-2 or Ark-6 (Table 1).

Two factors that can contribute to the discrepancy between gross and microscopic protection scoring are 1) the difference in onset of lesions after infection (damage is seen sooner microscopically) and bursal edema (if an IBD virus causes subtle edema, acutely infected bursas may actually make the B:BW cut-off despite the loss of lymphocytes). Thus, subtle edema could have made some AL-2 challenged bursas look grossly intact when they were in fact significantly depleted.

In the Lasher challenge study, protection was assessed strictly based on gross evaluation of bursas. This lab considers a 20% score as indicative of poor immunity to a challenge virus. Using this standard, the conventional program gave poor protection (20%) against the AL-10 virus (Table 2). According to sequencing analysis and monoclonal antibody reactivities, this virus is antigenically unique (9).

To summarize, all four conventional BTO vaccine programs produced solid Del-E B:BW protection. However, protection against the other four viruses in these challenge studies was lower, indicating lesser

neutralization by Del-E antibodies. This shows the capability of certain New-type viruses to infect flocks at an earlier age and, thus, potentially cause more IBD-related problems. These studies also showed that the substitution of just one of the two BTO vaccines with a Branch-1 containing vaccine resulted in equal or better Del-E protection and better cross-protection against all four New-type variant viruses studied. This difference was more exaggerated when judging protection on the microscopic level. Of the four New-type viruses, only Ark-6 is known to be a Branch-1 IBDV. The AL viruses have not yet been characterized according to phylogenetic analysis.

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