Report of the Miscellaneous and Toxic Diseases Subcommittee of the AAAP Information Paper Regarding Infectious Bursal Disease Virus

This paper is a summary of research work performed by Drs. Y. M. Saif, J. K. Rosenberger and P. D. Lukert which was presented in the form of oral presentations and the discussion that followed at the meeting of the Miscellaneous and Toxic Subcommittee on 21 July 1986 at the Annual Meeting of the American Association of Avian Pathologists in Atlanta, Georgia.

Opening Statement from Dr. Saif, Chairman

Questions have come from the poultry industry regarding isolates of infectious bursal disease virus (IBDV) that have been made recently from the States of Delaware, Maryland, and Alabama. The purpose of the meeting was to discuss the status of these isolates from those scientists involved in investigation of these viruses. The questions raised refer to antigenic relatedness and cross protection that is provided by commercial vaccines that are currently available for Serotype I IBDV. These questions have arisen because of reports from these investigators of differences in the antigenic properties of the new isolates compared to the old IBDV isolates (Serotype I).

History

In 1984, Saif reported on the isolation of an IBDV, designated MD, from 7 day old broilers on the Delmarva Penninsula. These broilers had bursal lesions and maternal anitbodies against serotypes I and II IBD virus. The MD isolate was classified as serotype I but was only about 50% related to other IBDV Serotype I viruses.

In January 1985 a meeting was called involving 9 integrated poultry companies and the Universities of Delaware and Maryland. This meeting was called because of an increase in respiratory disease that had occurred in broilers in the Delmarva area. A decision was reached by researchers at the University of Delaware to place vaccinated SPF sentinel birds on farms with a history of disease problems in hopes that viruses could be isolated that were responsible for the increase in disease. SPF sentinel birds were immunized at 1 day of age by subcutaneous injection with Bursal-Vac M along with vaccination for IBV, NDV, and reovirus. Two weeks following vaccination these sentinels were placed on problem farms at weekly intervals throughout the growout period. Beginning with the third week during the grow-out lesions were observed in the bursae of the vaccinated sentinel chickens. These lesions consisted of a rapid bursal atrophy with little to none of the gelatinous transudate associated with classical IBDV. Bursae were collected, homogenized, and the homogenate then inoculated into vaccinated sentinel chickens. Again lesions were observed and bursae were again collected. These bursae were homogenized and the homogenate treated with anti-serum to the serotype I IBDV. Following serum treatment the material was inoculated into SPF embryonated eggs with embryos showing a marked liver necrosis and splenomegaly 7 days PI. The isolates so obtained depending on whose laboratory the virus was isolated in have been named A, D, E and G (Rosenberger), Md (SAIF), Ala, B and 51(Lukert). All of these

viruses may be the same or antigenically very similar but they are all antigenically different from the Serotypes I (conventional vaccine and standard challenge viruses) and Serotypes II IBD virus.

Methodology

All 3 scientists agreed that they had found these new isolates to be different but there was some variation in what each had found. These differences could possibly be related to the methodology each had used in studying the isolates.

Dr. Saif reported that his laboratory is using a constant virus (100 $TCID_{50}$) varying serum method for determining antigenic relatedness. This is a cell culture method involving the GM 70 established cell line and primary chicken embryo fibroblasts. The formula of Archetti and Horsfall is used to establish relatedness. Serotyping is based on the formula established by Kapikian et al. where a virus is considered to belong to a different serotype if 100 $TCID_{50}$ of that virus is not neutralized by 20 units of antibody to the prototype virus of a given serotype.

Dr. Lukert stated his laboratory is using a constant virus (100 $TCID_{50}$) varying serum method using bursal cells derived from 19 to 20 day old embryos. The indicater of infection is immunofluorescence using a fluorescein conjugated anti IBDV antibody made in chickens. Formulas used to compute relatedness are the same as Dr. Saif's method.

Dr. Rosenberger is using a varying virus constant serum method in 9 to 11 day old SPF embryonated eggs. Eggs are inoculated by the CAM route. A neutralization index is determined and it is based on evaluation of embryos for both lesions and mortality. The lesions produced by the new viruses include liver necrosis and splenomegaly while lesions in embryos with the standard challenge virus (Serotype I) is mainly subcutaneous hemorrhage along with embryo mortality. All egg evaluations are carried out 7 days post inoculation. In the embryo test 2 logs difference in the neutralization index is interpreted as an antigenic difference between 2 viruses.

Antisera used by Lukert in his procedure is produced in chickens and rabbits, Saif uses chicken and guinea pig antisera, while Rosenberger uses chicken antisera exclusively.

Neither Lukert or Saif have to this point performed cross protection studies. Dr. Rosenberger has performed several studies using 10^6 EID_{50} of formalin inactivated virus administered intramuscularly (IM) as an oil emulsion preparation. Chickens are immunized and then challenged 4 weeks later. Nine to ten days post challenge these birds are sacrificed and post mortemed. The bursa of each bird is examined for the presence of bursal atrophy, which is determined by calculating the bursal body weight ratios. If the bursal body weight ratio is 2 or more standard deviations away from the mean of nonchallenged controls the bursa is considered to be atrophic.

Results

The results of laboratory testing in Dr. Saif's laboratory are that there are at least 3 groups of IBD viruses belonging to Serotype I that are antigenically different. These groups include some vaccine strains and a recent isolate (Md) from the Delmarva Peninsula.

The results from Dr. Lukerts' laboratory show that the new isolates from Delmarva and Alabama show marked antigenic differences from several serotype I vaccine viruses and a serotype II virus (MO).

The results from Dr. Rosenbergers' laboratory on 5 of the recently isolated viruses from Delmarva and Alabama demonstrate marked differences antigenically both by cross neutralization and cross challenge from several commercially available IBDV vaccine strains and the USDA standard challenge virus. Vaccines made from the new viruses cross protected when birds were challenged with the USDA standard challenge virus but there was variable cross protection when birds were vaccinated with several existing commercial vaccines and then challenged with the new isolates.

Recommendations

The committee discussed the possibility of naming a prototype Serotype I IBD virus but no agreement could be reached for lack of full characterization of the proposed viruses.

The committee made several recommendations concerning future needs for research with the new IBDV isolates. These recommendations are as follows:

1. Does passage in cell culture change the following?
   a. antigenicity
   b. protective properties
   c. pathogenicity
2. Continued programs of monitoring for different antigenic strains of IBDV.
3. Continued research on the antigenic relatedness between old and the new isolates or strains of IBDV.
4. Characterization of the new isolates in the chicken.
5. Development of laboratory procedures that are specific and sensitive in detection of different antigenic types.
6. Development of modified live vaccines with broad antigenic properties.
7. Development of inactivated vaccines with broad antigenic properties.
8. The use of Kapikian et al. formula for serotyping and that of Archetti and Horsefull for examining antigenic relatedness was recommended for use with IBDV.
9. Laboratories conducting virus neutralization tests for diagnostic or research purposes should be aware of the possibility that the choice of virus to be used in the test might affect the results obtained.

Drs. Saif, Lukert, and Rosenberger all agreed that it was very important to exchange viruses, antisera, and information on the new IBDV isolates so the questions raised regarding the significance of these isolates to the poultry industry can be resolved.