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Antibody Dependent Enhancement of Infectious Bronchitis Virus in Poultry

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Abstract

Avian infectious bronchitis (IB) is a coronavirus infection of chickens that causes respiratory disease and reproductive problems in chickens. Currently, there are vaccines that are effective against IB. However, new variants and strains of avian infectious bronchitis virus (IBV) routinely emerge. A vaccine that is not the same strain as the virus is not completely effective in protecting against other variants because the vaccine will not allow the host antibodies to completely neutralize the strain. This is a problem because it makes IB difficult to control and diagnose.

Antibody-dependent enhancement (ADE) is a phenomenon whereby non-neutralizing antibodies, or low levels of neutralizing antibodies, facilitate access into the host cell and allows either an enhanced viral infection or an increase in the severity of the clinical disease. This means the virus may create more variants that render current vaccines ineffective, created problems in diagnoses and may lead to more severity clinical disease. ADE has been found to occur with dengue virus and other viruses including some coronaviruses. This is a concern because COVID-19 is a human coronavirus and many vaccines have been developed, but variants routinely arise. ADE is thought to be a very important factor for developing new vaccines because vaccines that are not specific for a serotype could enhance viral infections. This would be the first work of looking at ADE on IBV to determine if ADE is occurring.

Approach / Methods

To demonstrate ADE with IBV an egg embryo model was used. The Massachusetts vaccine strain of IBV with its homologous antisera prepared in SPF chickens were employed. We performed the virus neutralization assay in embryonating eggs using the beta method (i.e., diluted sera and constant virus) utilizing one egg infectious dose 50% (EID_{50}) IBV. Forty-eight hours following inoculation of the 10-day-old embryos with the IBV-antibody preparations, chorioallantoic fluid was harvested for RT-PCR evaluation and embryos were removed from their shells and evaluated for signs and lesions of IBV infectivity.

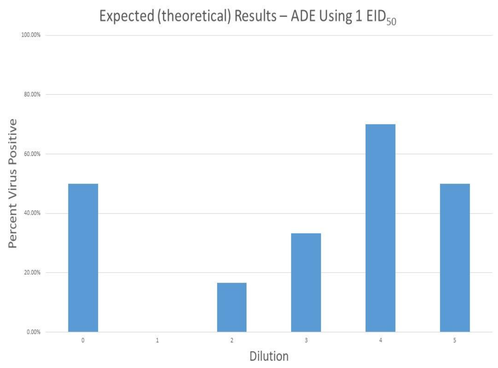

Figure 1. Theoretical results of ADE.

Introduction

Avian infectious bronchitis (IB) is a coronavirus infection of chickens causing respiratory disease and reproductive problems (i.e., decrease in egg production) and is a major problem for producers of broiler (meat-type) chickens and laying (egg-type) chickens. Currently, there are commercially available vaccines that are efficacious and safe. However, new variants and serotypes of avian infectious bronchitis virus (IBV) continue to emerge. A vaccine that is not the same serotype as the field virus will not protect against IBV infections or disease because the vaccine will not elicit from the host antibodies specific to neutralize the field strain. This is extremely problematic for the producer because it makes IB difficult to diagnose and control.

Antibody-dependent enhancement (ADE) is a phenomenon whereby non-neutralizing antibodies, or low levels of neutralizing antibodies, facilitate entry into the host cell and allow either an enhanced viral infection or a more severe clinical disease. ADE allows the virus a selective advantage and provides for new variants (and serotypes) to emerge. This phenomenon has been documented with several viruses including dengue virus (and other flaviviruses), influenza viruses and coronaviruses. More recently, ADE has been documented to occur with human coronaviruses and is a concern with COVID-19. ADE is thought to be a very important consideration for developing new vaccines because vaccines that elicit non-neutralizing antibodies could enhance viral infections and also give rise to new emerging virus serotypes. ADE has not been explored with IBV. The focus of this study was to determine if ADE occurs in IBV infections.

Results / Evaluation

A theoretical model of ADE occurring in embryos would show a complete neutralization at high antibody levels with a gradual increase as antibody levels decrease. Enhancement would be seen by a higher rate of infectivity than the control group, followed by a decrease back to control levels. This is illustrated in Figure 1. Trial one consisted of five control SPF eggs to determine the amount of virus needed to infect fifty percent of the eggs. There are four sample groups each consisting of twelve eggs. The antibody count for the sample groups are from high antibody concentrations to low antibody concentrations with respect to groups one through four. The control group (with no antibodies) had a percent positivity rate of 60.00%. Group one had a percent positivity of 16.67%, group two had a percent positivity of 33.33%, group three had a percent positivity rate of 75.00%, and group four had a percent positivity rate of 41.67%. The sample size for this trial was determined too small for statistical analysis. The results of trial one (figure 2) were nearly identical to the theoretical results (figure 1). This was repeated using more eggs with trial two. Trial two consisted of twenty eggs per sample group. The control group had a percent positivity of 70%. Group one had a percent positivity of 0.00%, group two had a percent positivity of 20.00%, group three had a percent positivity of 90%, and group four had a percent positivity of 95% which was statistically significant ($p<0.05$ by chi square test). The trial two results also appear to confirm the ADE theory but more dilutions would have been needed to observe the decrease in percent viral positivity that would be expected according to the theoretical model. We are conducting additional trials employing greater numbers of embryos and more serum antibody dilutions to further validate our results.

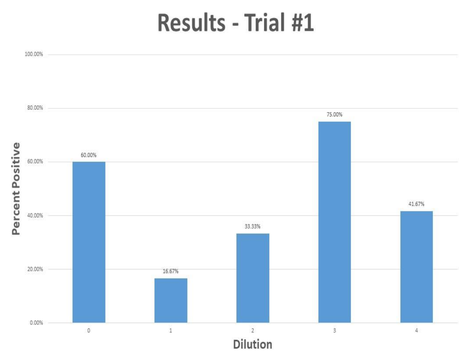

Figure 2. Results of Trial 1.

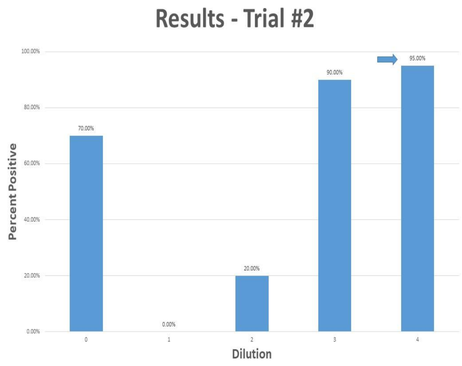

Figure 3. Results of Trial 2. Arrow indicates statistical significance ($p<0.05$)

Conclusion

The results of the above trials indicate that Antibody Dependent Enhancement (ADE) occurs with Infectious Bronchitis Virus in an egg embryo model. Our results indicate that using non-neutralizing amounts of homologous antibody demonstrated an increase in viral infectivity in chick embryos. This increase was predicted by our ADE model and reveals the potential danger of increased susceptibility of the host to viral infections when possessing low levels of antibody. More numbers are needed to increase statistical significance. The model needs to be tested with different IBV strain/antibody combinations. This would further validate ADE in a heterologous model. This is an important aspect because it could explain why vaccination against a specific virus strain could cause a more severe reaction and infection when confronted by a different strain. It also might explain the emergence of new variants in the wild. We believe that ADE merits consideration when selecting vaccines so that a viral outbreak is not exacerbated. Further testing of ADE could allow health experts to determine the correct amount vaccine/booster schedule to maximize protection against viruses.