Title: Development of an ELISA to Detect Serotype-Specific Antibodies against Infectious Bronchitis Virus

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Industry Summary

Infectious bronchitis (IB), caused by infectious bronchitis virus (IBV) is an acute, highly contagious viral respiratory disease and is one of the most common and economically important diseases in the poultry industry. The U.S. poultry industry looses an estimated 20 million dollars annually to IB even though every broiler in the U.S. has been vaccinated against IB. The reason for continuous problems with IB is that many serotypes of IBV have been involved in the disease and there is little or no cross protection among different serotypes.

The spike protein (S), which is cleaved into the S1 and S2 subunits, of IBV has been identified to play an important role in inducing protective immunity. Determination of the serotype that causes the disease is also based on the structure of the S1 protein. An enzyme-like immunosorbent assay (ELISA) test based on the presence of antibody against the serotype-specific S or S1 proteins in the diseased birds would help to diagnose and type IBV more rapidly and accurately. Therefore, the information would be beneficial for the industry to know which vaccine strain(s) should be included to protect flocks against field strains of IBV challenges.

The overall objective was to develop an ELISA assay that can be used to detect a specific serotype of IBV. The specific aims of this study were 1) to evaluate whether chicken-origin cells are suitable for producing avian viral proteins from adenovirus recombinants, and 2) to produce antigens that can be used in ELISA to differentiate different serotypes of IBV.

Our results indicated that adenovirus could infect and replicate in a chicken hepatoma LMH cell line as well as a human 293 cell line. This was the first report that human adenovirus can be used as a vector to infect a chicken cell line; therefore, an avian viral antigen can be correctly produced in a chicken cell line. The objective 1 was achieved. In objective 2, a total five recombinant adenovirus clones that carry the serotype-specific antigens were constructed. All clones were transfected into 293 cells to produce the Arkansas serotype antigens, respectively. The expression level of the S protein was determined using western blotting with monoclonal and polyclonal antibodies specific to the Arkansas serotype. However, the expression of the S or S1 protein was either weak or could not be detected in either 293 or LMH cell lines. The objective 2 was completed but the antigen could not be produced sufficiently to be used for an ELISA assay.

There is no immediately impact for the industry. However, a chicken-origin LMH cell line is proved to be suitable for producing chicken-origin viral antigen in an adenovirus expression system.
**Scientific Report**

Infectious bronchitis (IB), caused by infectious bronchitis virus (IBV) is an acute, highly contagious viral respiratory disease and is one of the most common and economically important diseases in the poultry industry. The U.S. poultry industry loses an estimated 20 million dollars annually to IB even though every broiler in the U.S. has been vaccinated against IB. The reason for continuous problems with IB is that many serotypes of IBV have been involved in the disease and there is little or no cross protection among different serotypes.

The spike protein (S), which is cleaved posttranslationally to release S1 and S2 subunits, of IBV has been identified to play an important role in inducing protective immunity. The serotype-specific epitopes appear to be on the S1 subunit. Analysis of amino acid sequences revealed that considerable heterogeneity existed among the S1 subunits of different strains and isolates of IBV. An enzyme-like immunosorbent assay (ELISA) test based on the presence of antibody against the serotype-specific S or S1 proteins in the birds would help to diagnose and type IBV more rapidly and accurately. Therefore, the information would be beneficial for the industry to know which vaccine strain(s) should be included to vaccinate the birds.

The overall objective of this study was to develop a serotype-specific serologic assay to detect IBV. Since the Arkansas type (Ark) of IBV was one of the most common serotypes isolated in the southeastern U.S., the Ark serotype was used as a model. The specific aims of this study were 1) to determine if the adenovirus vector 5 (Ad5) can replicate in chicken embryonic kidney cells and a continuous line of chicken hepatoma (LMH) cells, 2) to evaluate if the recombinant Ad5 can be used to express S protein of IBV.

In objective 1, the infection of replication-defective human adenovirus type 5 vector (Ad5) in primary chicken embryonic kidney (CEK) cells or a continuous line of chicken hepatoma (LMH) cells was tested. The results showed that Ad5 could infect both CEK and LMH cells, but only LMH cells could support the replication of Ad5 (Figures 1 and 2). Our study suggests that Ad5 could be propagated in LMH cells as well as in an Ad5-transformed human embryonic kidney (293) cell line (Figures 2 and 3). This result is significant because it is the first time to prove that Ad5 could replicate in the chicken cell line. The cell line of LMH probably is the better candidate for Ad5 expressing proteins derived from chicken because post-translational modification between mammalian and chicken cells are different.

In objective 2, five recombinant adenovirus clones were successfully constructed and the insert of S1 or S were confirmed (Figure 4). Clones 1 and 2 carried the S gene, Clone 3 carried the S1 gene, Clone 4 carried the S gene with histidine tag, and Clone 5 carried the S gene with histidine tag. All clones were transfected into 293 cells. The expression level of the S or S1 protein was determined using western blotting with monoclonal and polyclonal antibodies specific to the Arkansas serotype. However, the expression of the S or S1 protein could not be detected suggesting that either the S and S1
protein could not be expressed or expressed at low level. Another explanation is that the antigenicity of the S or S1 protein is conformational dependent; the S or S1 protein may be produced but can not be recognized by the antibodies used in this study.

Figure 1. Dose responses and kinetics of Ad5 infection in CEF cells

Figure 2. Dose responses and kinetics of Ad5 infection in LMH cells
Figure 3. Dose responses and kinetics of Ad5 infection in 293 cells

Figure 4. (A) Screening of recombinant AdEasy-1 plasmid with restriction enzyme. Lanes 1, 1 Kb DNA ladder; lanes 2 and 3, recombinant AdEasy-1 plasmids, from colonies 23 and 27, respectively, digested with Pac I, lane 4, recombinant pShuttle-CMV digested with Kpn I and Hind III, Lanes 5 and 6, recombinant AdEasy-1 plasmids, from
colonies 23 and 27, respectively, digested with Kpn I and Hind III. The restriction fragments indicated the presence of an insert in an adenovirus vector. (B) PCR analysis confirmed the presence of the inserted gene encoding for the S or S1 protein.

Presentations and Publications: