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Short Paper

Development of a recombinant protein-based dot-blot hybridization assay for the detection of antibody to chicken infectious bronchitis virus

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Summary

Nucleocapsid (N) protein of infectious bronchitis virus (IBV), one of the viral structural proteins, induces strong antibody response in natural infection. In this study, a simple, recombinant N protein-based dot-blot test was developed to serologically examine chicken serum samples for the presence of IBV antibody. Initially, 72 serum samples were tested for the presence of IBV antibody using a commercial enzyme linked immunosorbent assay (ELISA) kit. Forty six IBV positive serum samples (group A) produced strong signals in the dot-blot assay. Seven negative serum samples (group B) produced weak but specific signals using the dot-blot assay in conjunction with Western blot analysis. The remaining 19 samples (group C) from IBV negative specific pathogen free (SPF) chickens did not produce visible signals using the dot-blot assay. In conclusion, the above results suggest that the dot-blot assay is a reliable, sensitive, and specific test for serological detection of IBV positive chickens.

Key words: Infectious bronchitis virus, Dot-blot hybridization, Nucleocapsid protein

Introduction

Infectious bronchitis (IB) is an acute, highly contagious upper respiratory disease of chickens (Cavanagh and Naqi, 2003). Tracheal rales, sneezing and coughing are the major clinical signs in the young chickens. Infectious bronchitis has been diagnosed in Iranian chicken flocks with molecular, serological, and virus isolation methods (Aghakhan *et al*., 1994; Haqshenas *et al*., 2005; Mahzounieh *et al*., 2006). Despite the wide use of live attenuated and inactivated vaccines to protect commercial chicken flocks, IB remains responsible for serious financial losses to the poultry industry of the country.

Infectious bronchitis virus (IBV) encodes three major structural proteins; the spike (S) glycoprotein, the integral membrane glycoprotein (M), and the nucleocapsid phosphoprotein (N) (Cavanagh, 1981; Spaan *et al*., 1988; Saif, 1993). The N protein is an immunodominant antigen inducing cross-reactive antibodies in high titres (Ignjatovic and Galli, 1993). Recently, we have cloned and bacterially expressed the complete N gene of IBV (Haqshenas *et al*., 2004). Here, for the first time, we tested whether the recombinant N protein of the H120 strain of IBV can be used to specifically detect antibodies to the virus.

Materials and Methods

Preparation of the recombinant N protein

The N protein of IBV was bacterially expressed and purified as described

previously (Haqshenas *et al*., 2004). The purified protein was quantified using the Bradford method (Bradford, 1976).

Enzyme linked immunosorbent assay (ELISA)

Seventy two serum samples were received from three different chicken farms by Iranian Veterinary Organization (IVO). The negative control serum samples were collected from specific pathogen free (SPF) chickens. All serum samples were tested for the presence of IBV antibody using an IDEXX IBV commercial ELISA kit according to the manufacturer's instruction (IDEXX, Westbrook, ME).

Dot-blot hybridization assay

Dot-blot hybridization assay was carried out according to standard protocols (Sambrook and Russell, 2001). Following preliminary optimization of different concentrations of the recombinant N protein and primary antibody (data not shown), 25 ng of antigen was used per each dot onto PVDF Western Blotting Membrane (Roche Diagnostics GmbH, USA), and the primary antibody was diluted 1/200. Western blotting was performed as described previously (Haqshenas *el al.*, 2004). Infectious bronchitis virus positive and negative serum samples from our previous study (Haqshenas *et al*., 2004) were used as control samples.

Western blot analysis

SDS-PAGE was used to demonstrate whether the serum samples categorized as group B specifically reacted with the recombinant N protein. For each serum sample, 500 ng of the purified recombinant N protein was resolved onto a 12% polyacrylamide gel using SDS-PAGE. The protein was subsequently trans-blotted onto a PVDF Western Blotting Membrane using standard protocols (Sambrook and Russell, 2001). The membranes were cut into strips, and each strip was used to test one serum sample as described for dot-blot hybridization.

Results

ELISA

All serum samples were tested by the IVO using a commercial ELISA kit as described in Materials and Methods. Forty six serum samples (group A) produced ODs above the cutoff and they were reported positive. Seven serum samples (group B) produced ODs above the negative control but lower than cutoff. All nineteen serum samples (group C) from SPF chickens were negative with ODs similar to negative control.

Dot-blot hybridization assay

When a serum sample produced a signal stronger than negative control, it was reported as positive. Using the dot-blot assay all the serum samples from group A generated strong visible signals on the membrane (Fig. 1A) suggesting a good correlation between the ELISA and dot-blot assay. None of the samples from group C created a visible signal in the dot-blot assay (Fig. 1B). However, the remaining seven samples (group B) that produced ODs less than the cutoff but above the negative sample generated very weak signals using Western blot analysis (Fig. 2). The signals of four samples of this group were easily detected. To demonstrate that the dot-blot signals produced by group B samples were specific, combination of SDS-PAGE and immunoblot analysis were used. These assays showed that all the serum samples of group B specifically reacted with the over-expressed protein bands (Fig. 2) although the signals of the two (number 5 and 7) were very weak. As described previously, the recombinant N protein was observed as two protein bands in immunoblot analysis.

Discussion

Chicken IB leads to a wide range of clinical signs in young and layer chickens, and it is responsible for high economical losses in poultry industry worldwide (Cavanagh and Naqi, 2003). So far, many IBV serotypes have been identified (Ignjatovic and Sapats, 2000). The recombinant N protein of IBV has been used

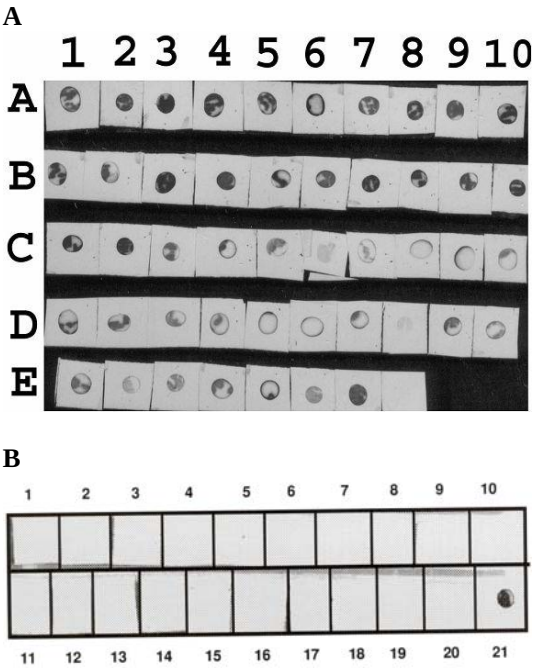

Fig. 1: (A) Detection of IBV-positive sera by the dot-blot hybridization assay. E7 and E8 acted as positive and negative controls, respectively. (B) Examination of 19 IBV negative serum samples from SPF chickens. Serum samples 20 and 21 acted as negative and positive controls, respectively

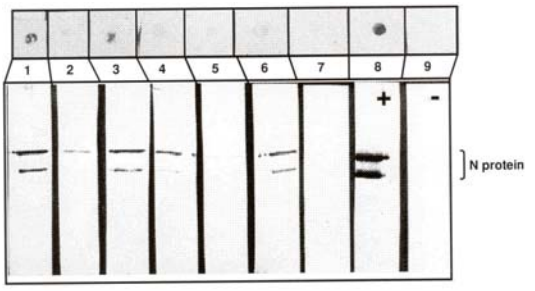

Fig. 2: Dot-blot hybridization assay and SDS-PAGE and immunoblot analysis. Top: Serum samples that produced ODs below the cutoff but above negative control were tested using the dot-blot assay. Bottom: The specificity of the dot-blot signals were evaluated using the combination of SDS-PAGE and immunoblot analysis. Lanes 8 and 9 represent positive and negative controls, respectively

to develop various diagnostic tests (Ignjatovic and Galli, 1993; Ndifuna *et al*., 1998). The N protein used in this study is highly conserved and shares 94-99% identity among different IBV strains (Sneed *et al*., 1989; Williams *et al*., 1992). Therefore, the recombinant N protein can be used as a group-specific protein to detect IBV infected chickens, and to evaluate the immunological response of chickens to IBV vaccine. It has been reported that the recombinant N protein of Gray strain of IBV could be used to detect antibody to different strains of IBV (Ndifuna *et al*., 1998). IBV antibody is currently detected by the use of a commercial ELISA, which is very expensive, and needs specific equipment such as ELISA reader. In the current study, it was demonstrated that the recombinant N protein of strain H120 can be used to develop a simple, reliable, and sensitive dot-blot hybridization assay for the detection of anti-IBV antibody. As indicated in the results section, seven serum samples (group B) that were detected IBV negative using the commercial ELISA produced weak signals using the dot-blot assay. This could be due to different dilutions of serum samples that were used (1/500 dilutions for ELISA *vs* 1/200 dilutions for the dot-blot assay). Furthermore, as mentioned in the results section, the ELISA ODs of these serum samples were above the negative samples. Combination of SDS-PAGE and immunoblot analysis revealed that the dot-blot signals of these seven samples were specific. On the other hand, none of the samples obtained from SPF chickens generated a visible signal on the membrane. Collectively, the above results indicate that the specificity and sensitivity of dot-blot assay are comparable to the commercial ELISA. To further examine the specificity of the dot-blot test, we also demonstrated that serum samples containing high antibody titers to Newcastle disease virus, infectious bursal disease virus, and influenza virus did not react with the recombinant N protein.

Where the ELISA equipments are available, this test is a preferential test to perform for the detection IBV-positive serum samples. However, the equipments used in ELISA test are expensive and may not be affordable by some laboratories. Therefore, we believe that the in-house dot-blot hybridization assay described in this manuscript will be a simple, in-expensive, and reliable test for the detection of IBV natural infections as well as for the evaluation of immune response following IBV vaccination.

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