# IMPROVEMENT OF IMMUNODIAGNOSIS OF AVIAN INFECTIOUS BRONCHITIS VIRUS (IBV) INFECTION BY THE DETECTION OF IgM AND IgG ANTIBODIES USING A NEW RECOMBINANT NUCLEOPROTEIN-BASED ELISA

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## ABSTRACT

Conventional serology of IBV infection could be improved, by demonstrating a seroconversion, using paired serum sets or the detection of IBV-specific immunoglobulin M (IgM) raise, and by replacing the indirect ELISA procedure based on the use of microplates coated with purified whole virus particles by recombinant viral proteins. In this study we describe a new indirect ELISA (Nrec-ELISA) that uses recombinant Nucleoprotein (N) for the detection of IBV-specific IgM and IgG antibodies in chicken sera. The Nrec-ELISA showed in IBV experimentally infected birds an early seroconversion for IgM antibodies, which declined rapid, whereas IgG anti-IBV antibodies appeared latter, but persisted longer than IgM. Coincident positive (IgM+/IgG+) or negative (IgM-/IgG-) results were detected for 48.91% and 20.35%, respectively, of serum samples collected from chickens naturally infected with IBV. However, discordant, IgM+IgG- or IgM-/IgG+ results, were identified for 22.08% and 8.66% of the samples, suggesting that these latter birds were in acute or late phase of IBV infection, respectively. These results indicate that the chickens infected with IBV produced distinct antibody levels of IgM and IgG isotypes, in acute and late infection phases, and these antibody isotypes reacted strongly with the recombinant N protein expressed in E. coli. In conclusion, the ELISA performed with the recombinant N protein can be used for the detection of antibodies, especially of IgM isotype, in IBV infected birds, with the advantage of detecting early the humoral immune responses.

**KEY-WORDS:** birds, recombinant N protein, humoral immune responses and ELISA.

## INTRODUCTION

The avian infectious bronchitis virus (IBV) is the etiologic agent of infectious bronchitis, which causes an acute, highly contagious disease in chickens, affecting the respiratory, reproductive and renal systems. IBV is distributed worldwide and impacts considerably the poultry industry (Cavanagh, 2007). IBV is enveloped virus with a single stranded RNA of positive polarity, approximately 27,6 kb in length (2) which encodes four major structural proteins: the spike protein (S), the membrane protein (M), the envelop protein (E), and the nucleocapsid protein (N) (Cavanagh, 2007). The N

protein contained 409 amino acids and is highly conserved among different IBV isolates with 91-96.5% similarity among various IBV strains (1) (Seah et al., 2000).
Rapid diagnosis and determination of flock immune status is critical in controlling outbreaks of IBV that result in severe economic losses in breeder, layer and broiler poultries (De Wit, 2000). Enzyme immunoassays have been proved accurate indicators of antibody levels against IBV and facilitate the monitoring of immune status in large poultry flocks (Snyder et al., 1983).

The recombinant nucleoprotein of IBV was expressed in prokaryotic (*Escherichia coli*), yeast (*Saccharomyces cerevisiae*), and successfully used as antigen in an ELISA (Ndifuna et al, 1998, Gibertoni et al, 2005). Acute IBV infections may be diagnosed by direct methods like virus isolation and gene detection by RT-PCR techniques, or by indirect methods such as the immunodiagnostic tests (De Wit, 2000). Usually, a seroconversion using paired serum sets is done for IB diagnosis. However, the paired sera evaluation is expensive and impractical as it is time-consuming and more labor-intensive (De Wit, 2000). The alternative is to detect IBV-specific IgM antibodies, since these antibodies are only present temporally after infection or the administration of live vaccines against IBV (De Wit, 2000). In this study we describe a method of indirect ELISA with a recombinant nucleoprotein of IBV as antigen (N-Rec-ELISA) for detecting IBV-specific antibodies of IgM and IgG isotypes in serum samples from birds experimentally infected, or birds from poultry flocks suspected of infection with this virus.

## MATERIAL AND METHODS

**Standardization of N-Rec-ELISA:** Firstly, different dilutions of purified preparation of N recombinant protein produced in *Escherichia coli* pET28a expression system (Novagen) and IBV -positive and IBV-negative chicken sera were determined by checkerboard titration in the N recombinant protein ELISA (N-Rec-ELISA), for the detection of IBV-specific of IgM and IgG antibodies . The N-Rec-ELISA followed the general recommedation of Gibertoni, et al.(2005).

**Experimental Protocol I – Kinectics of IgG and IgM anti-IBV antibody responses in Infected Chickens:** One group of twelve specific-pathogen-free (SPF) White-Leghorn chickens was housed in positive pressure isolators up to 28 days of age and challenged with intranasally and intraocularly with $10^{5.0}$ EID of a virulent M41 strain of IBV. Serum samples were collected at 5, 10, 15 and 20 days post-infection (p.i) to measure anti-IBV IgG and IgM antibody levels by an indirect ELISA using a recombinant nucleoprotein expressed in *E. coli* as coating antigen.

**Detection of Anti-IBV IgG and IgM Antibodies in field Serum Samples:** A total of 231 serum samples were collected from broilers and layer commercial poultries, during the clinical, acute phase of an IBV infection. The serum samples were separated into 3 groups based on the presence of infection by IBV and on the number of vaccinations against this virus, e.g., group 1 contained non-vaccinated birds, group 2 contained 1x IBV vaccinated birds and group 3 contained ≥2x vaccinated birds. The IBV infections were diagnosed by nested RT-PCR using primers to amplify a fragment of 3'- untranslated region of IBV.

## RESULTS AND DISCUSSION

IBV-positive and negative chicken sera were more accurately distinguished in the blocking-titration of N-Rec-ELISA, at an antigen concentration of 0.125 ug/ml and at serum dilution of 1:800 for anti-IBV IgG antibodies and a concentration of 0.5ug/ml and a serum dilution of 1:400 for anti-IBV IgM antibodies (Fig. 1). The determination of the kinectics profile of anti-IBV antibodies in SPF chickens experimentally infected with M41 strain of IBV (Fig. 2) showed that IgM anti-IBV antibodies raised early, i.e., from 5 to 10 days p.i., in the experimentally infected birds, and started to decline early (from 15 to 20 days p.i). On the contrary, the IgG antibodies appeared latter and maintained at high levels up to 20 days p.i., in the infected birds. The analysis of twelve samples of IBV-negative sera from non-infected and non-vaccinated SPF chickens (negative control group) revealed cut-off points equivalent to the values S/P of 0.079 and 0.060 (value average S/P ± 3 standard deviations), respectively for the N-Rec-IgM-ELISA and N-Rec-IgG-ELISA.

Coincident positive (IgM+/IgG+ / 48.91%) or negative (IgM-/IgG- / 20.35%) results were detected for serum samples of chickens naturally infected with IBV, especially those collected from groups 3 (≥2x IBV vaccinated chickens) and 1 (non-IBV vaccinated chickens), respectively. However, discordant results, IgM+IgG- (22.08%) or IgM-/IgG+ (8.66%) were also identified for samples of IBV infected chickens, especially those from groups 1 and 3, respectively.

The detection of a greater number of IgM + birds in group 1 is probably due to the occurrence of a more recent infection with IBV, which results in a primary production of anti-N IgM antibodies as detected in ELISA using purified preparations of IBV (MARTINS et al., 1991, De Wit et al., 1998 ). On the other hand, in the vaccinated birds from groups 2 and 3 an evident discrepancy, favoring a great detection of IgM + birds, was not found, probably because a memory for the humoral immune response has already developed after IBV infection on these birds, especially for IgG antibody response.

## CONCLUSION

The rapid and short-lived IgM anti-IBV nucleoprotein antibody responses can be accurately detected by N-Rec-ELISA and used as indicative for acute IBV infections, contributing to the early diagnosis of IB infection.

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