

# Expression Of Nucleocapsid Gene Of Chicken Infection Bronchitis Virus Strain Massachuset H120

Mehragha, M.<sup>1</sup> and Seyfi Abad Shapouri, M.R.<sup>2</sup>

Received: 20.01.2017

Accepted: 28.10.2017

## Abstract

Infectious bronchitis (IB) is one of the most important viral diseases of poultry that causes significant economic losses to chicken industry worldwide. Infectious Bronchitis Virus causes a contagious and acute respiratory disease. Some nephrotropic serotypes of IBV cause nephritis. IBV is responsible for decrease of egg production and laying abnormal eggs. IB has no treatment and the only way to protect the chicken flocks is vaccination against the virus. Immunity to IBV has most often been assessed using serological assays; like enzyme-linked immunosorbent assay (ELISA). IBV (genus *Gamacoronavirus*, family *Coronaviridae*) contains a 27.6 kb single stranded positive sense RNA. The genome encodes three major structural proteins; the spike (S) glycoprotein, the integral membrane glycoprotein (M), and the 43 to 50 kDa nucleocapsid (N) phosphoprotein. The aim of this study was to prepare recombinant nucleocapsid protein. For this purpose after RNA extraction the corresponding gene with a length of 1227 bp was amplified by RT-PCR. Then this gene cloned into expression plasmid (pMAL-C<sub>2</sub>X) and recombinant plasmid was transferred to strain Rosetta of *E.coli*. Expression of protein N was detected by SDS-PAGE. The results of this study show Nucleocapsid recombinant protein could be expression with high concentration in bacterial system.

**Key words:** Infectious bronchitis virus, Nucleocapsid Protein, Expression

---

1- DVM Graduated from Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

2- Professor, Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

**Corresponding Author:** Mehragha, M., E-mail: mrymehragha@gmail.com

## References

- Abdel-Moneim, A.S.; El-Kady, M.F.; Ladman, B.S. and Gelb, J. Jr. (2006). S1 gene sequence analysis of a nephropathogenic strain of avian infectious bronchitis virus in Egypt. *Virology Journal*, 3: 1-9.
- Aghakhan, SM.; Abshar, N.; Fereidouni, SR.; Marunesi, C. and Khodashenas, M. (1994). Studies on avian viral infections in Iran, *Archives of Razi Institute*, 44: 1-5.
- Anjum, AD. (1997). *Poultry Diseases*. 2<sup>th</sup> ed. Veterinary Ag Publication, Faisalabad, Pakistan. Pp:24-29.
- Ausubel, F.M.; Brent, R.; Kingston, RE.; Moore, D.D.; Seidman, J.G.; Smith, J.A. and Struhl, K. (1992). *Short Protocols in Molecular Biology*. 2<sup>nd</sup> ed., John Wiley and Sons, New York. Pp: A1-15.
- Boots, A.M.H.; Benaissa-Trouw, B.J.; Hesselink, W.; Rijke, E.; Schrier, C. and Hensen, E.J. (1992). Induction of anti-viral immune responses by immunization with recombination-DNA encoded avian coronavirus nucleocapsid protein. *Vaccine*, 10 (2): 119-124.
- Cavanagh, D. (1997). Nidovirales: a new order comprising Coronaviridae and Arteriviridae. *Archive of Virology*, 142 (3): 629-633.
- Compton, S.R.; Rogers, D.B.; Holmes, K.V.; Fertsch, D.; Remenick, J. and McGowan, J.J. (1987). In vitro replication of mouse hepatitis virus strain A59. *Virology*, 61(6): 1814-1820.
- Emikpe, B.O.; Ohore, O.G.; Olujonwo, M. and Akpavie, S.O. (2010). Prevalence of antibodies to infectious bronchitis virus (IBV) in chickens in Southwestern Nigeria. *African Journal Microbiology Research*, 4(1): 092-095.
- Gibertoni, A.M.; Montassier, M. de F.; Sena, J.A.; Givisiez, P.E.; Furuyama, C.R. and Montassier, H.J. (2005). Development and application of a *Saccharomyces cerevisiae* expressed nucleocapsid protein-based enzyme-linked immunosorbent assay for detection of antibodies against infectious bronchitis virus. *Journal of Clinical Microbiology*, 43(4): 1982-1984.
- Huang, Y.P.; Lee, H.C.; Cheng, M.C. and Wang, C.H. (2004). S1 and N gene analysis of avian infectious bronchitis viruses in Taiwan. *Avian Disease*, 48(3): 581-589.
- Ignjatovic, J. and Galli, L. (1993). Structural proteins of avian infectious bronchitis virus: role in immunity and protection. [Advances in Experimental Medicine and Biology - Springer](#), 342: 449-453.
- Ignjatovic, J. and Galli, L. (1993). Structural proteins of avian infectious bronchitis virus: role in immunity and protection. *Advance Experimental Medical Biology*, 342: 449-453.
- Jackwood, MW.; Hilt, DA.; Williams, SM.; Woolcock, P.; Cardona, C. and O'Connor, R. (2007). Molecular and serologic characterization, pathogenicity, and protection studies with infectious bronchitis virus field isolates from California. *Avian Diseases*, 51(2): 527-533.
- Kant, A.; Koch, G.; van Roozelaar, D.J.; Kusters, J.G.; Poelwijk, F.A. and van der Zeijst, B.A. (1992). Location of antigenic sites defined by neutralizing monoclonal antibodies on the S1 avian infectious bronchitis virus glycopolyptide. *General Virology*, 73(3): 591-596.
- Liu, S.W.; Zhang, Q.X.; Chen, J.D.; Han, Z.X.; Liu, X.; Feng, L. et al. (2006). Genetic diversity of avian infectious bronchitis coronavirus strains isolated in China between 1995 and 2004. *Archive of Virology*, 151(6): 1133-1148.
- MacLachlan, N.; James, and Dubovi, Edward J. (2011). *Fenner's Veterinary Virology*, 4<sup>th</sup> ed., Amsterdam, Pp: 16-19.
- Marquardt, W.W.; Snyder, D.B. and Schlotthober, B.A. (1981). Detection and quantification of antibodies to infectious bronchitis virus by enzyme-linked immunosorbent assay. *Avian Disease*, 25(3): 713-722.
- Mockett, A.P.A. and Darbyshire, J.H. (1981). Comparative studies with an enzyme-linked immunosorbent assay (Elisa) for antibodies to avian infectious bronchitis virus. *Avian Pathology*, 10(1): 1-10.
- Ndifuna, A.; Waters, A.K.; Zhou, M. and Collisson, E.W. (1998). Recombinant nucleocapsid protein is potentially an inexpensive, effective serodiagnostic reagent for infectious bronchitis virus. *Virological Methods*, 70(1): 37-44.

- Niesters, H.G.; Lenstra, J.A.; Spaan, W.J.; Zijderveld, A.J.; Bleumink-Pluym, N.M.; Hong, F. et al. (1986). The peplomer protein sequence of the M41 strain of coronavirus IBV and its comparison with Beaudette strains. *Virus Research*, 5(2-3): 253-263.
- Sang Heui, Seo.; Jianwu, Pei.; W. Elwood, Briles.; Jennifer, Dzielawa. and Ellen, W. Collisson. (2000). Adoptive Transfer of Infectious Bronchitis Virus Primed alphabeta T Cells Bearing CD8 Antigen Protects Chicks from Acute Infection. *Virology*, 269(1): 183-189.
- Schalk, A.F. and Hawn, M.C. (1931). An apparently new respiratory disease of baby chicks. *Journal of the American Veterinary Medical Association*, 78: 413-422.
- Seify abad Shapouri, M.R.; Mayahi, M.; Charkhkar, S. and Assasi, K. (2002). Serotype identification of recent Iranian isolates of infectious bronchitis virus by type-specific multiplex RT-PCR. *Archives of Razi Institute*, 53: 79-85.
- Siddell, S.G. (1995). The coronaviridae: an introduction. In: Siddell, S.G. Jr. (Ed). *The Coronaviridae*. Plenum Press, New York, Pp: 1-10.
- Tripti, Jain.; Megha, Kadam.; Asit, Jain. and Sarkhel, B.C. (2013). Prokaryotic Expression of N Gene of Infectious Bronchitis Virus and Assessment of its Immunogenicity and Antigenicity. *Indian Journal of Animal Research*, 47(4): 292-300.
- Wang, C.H.; Hong, C.C. and Seak, J.C. (2002). An ELISA for antibodies against infectious bronchitis virus using an S1 spike polypeptide. *Veterinary Microbiology*, 85(4): 333-342.
- Williams, A.K.; Wang, L.; Sneed, L.W. and Collisson, E.W. (1992). Comparative analysis of the nucleocapsid genes of several strains of infectious bronchitis virus and other coronaviruses. *Virus Research*, 25(3): 213-222.
- Zhang, Jinliang; Guo, Yan.; Xiao, Yuncai.; Wang, Xiliang.; Zhonghua, Li.; Sishun, Hu. and Dingren, Bi. (2010). A Simple and Rapid Strip Test for Detection of Antibodies to Avian Infectious Bronchitis Virus. *Veterinary Medicine Science*, 72(7): 883-886.
- Zhou, M.; Williams, A.K.; Chung, S.I.; Wang, L. and Collisson, E.W. (1996). The infectious bronchitis virus nucleocapsid protein binds RNA sequences in the 3' terminus of the genome. *Virology*, 217: 191-199.