Cairo University

Faculty of Veterinary Medicine

Department of Virology

Cloning and Expression of BHV-1 outer surface protein genes

A Thesis presented by

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(B.V.Sc., Cairo University, 2005)

(M.V.SC. Virology, Cairo University, 2010)

For The Degree of Ph.D.V.Sc in Veterinary Medicine Science

(Virology)

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Title of thesis: Cloning and Expression of BHV-1 outer surface protein genes protein of BHV-1.

Abstract

The Glycoprotein D (gD) gene of bovine herpesvirus-1.1 Egyptian strain "Abu-Hammad" was cloned and expressed in Spodopetra Frugiperda (Sf9) insect cells using baculovirus expression system. Full length gD encoding sequence was amplified by polymerase chain reaction (PCR) and cloned into the baculovirus shuttle vector; pMelBac B. The cloned gene was inserted in the genome of Autographa California nuclear polyhydrosis virus (AcMNPV) under control of the polyhedrin promoter, through homologous recombination between the recombinant pMel/gD and a linearized triple cut baculovirus DNA (Bac-N-Blue) using liposome mediated transfection on to Sf9 cells. Recombinant baculovirus was selectively purified by plaque assay and verified for integrity of gD gene of BoHV-1.1 using PCR. The recombinant gD protein maintained their antigenic properties as determined by its reactivity with anti BoHV-1 positive serum using situ immunofluorescent and Western blot assays. The secreted recombinant gD in culture medium of infected insect cells was used as a coating antigen in an indirect enzyme linked immunosorbent assay (ELISA) to test its utility for detection of antibody against gD of BoHV-1. This ELISA was compared to standard virus neutralization test for detection of anti-BoHV-1antibody in a panal of bovine sera demonstrating closely correlated antibody titers in both. The developed indirect gD-ELISA was a reliable candidate for detection and validation of BoHV-1seropositive animals with high specificity.

Key Word: BoHV-1 – glycoprotein D – Baculovirus – Sf9 – ELISA

Dedication

To my great father .

To my fantastically supportive mom.

To my beloved husband and my sweetheart sons "Ahmed and Aser".

To my lovely sisters, brothers.

To my lovely family.

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First of all, I would like to express my all-embracing gratitude and praise to our merciful God ALLAH for unlimited and continuous blessing on me and for his unmitigated support.

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1. Introduction

Bovine Herpesvirus type 1 (BoHV-1), is a double-stranded DNA virus of the genus Varicellovirus in the Alphaherpesvirinae subfamily of the Herpesviridae family causing infectious bovine rhinotracheitis (IBR), infectious pustular vulvovaginitis (IPV) and infectious balanoposthitis (IBP) **(Gibbs and Rweyemamu, 1977; Schwyzer and Ackermann, 1996).**

Bovine herpesvirus 1 (BoHV-1), is an important contagious viral pathogen of domestic and wild bovines, is distributed worldwide exerting an economic impact on livestock industry. In Egypt, since 1960s attention was drawn to BoHV-1 (IBRV) as one the most significant causes of great economic losses in feedlot and dairy farms; mainly due to deaths form pneumoenteritis, mainly in cattle and buffalo calves, abortions, prolonged feeding periods with weight loss, costs of treatment and prevention programs. **Hafez and Fery, 1973** reported Incidence of the infectious and the virus was isolated by **Fatehya 1974**. Recently, some studies were reported on the molecular aspect of viruses **(El-Kholy, 2005 and Sobhy et al., 2014).**

BoHV-1 genome consists of a linear double stranded DNA molecule of about 136 Kilobases (Kb) long which is subdivided into a long unique region (UL) of 104 kbp and short region which contains a unique segment (US) of 10 kbp flanked by internal and terminal inverted repeats of 12 kb each **(Schwyzer & Ackerman 1996).** These repeats enable the US sequence to invert relative to the UL sequence, thus giving rise to two isomeric forms of the genome **(Farley et al., 1981; Mayfield et al., 1983).**

BoHV-1 nucleotide sequence encodes for at least 69 proteins among which 10 – 12 are glycoproteins. Because of their location in the virion envelope and on the surface of infected cells, glycoproteins are important targets for both molecular and immunological assays **(Schwyzer & Ackermann, 1996).**

Also, these glycoproteins are implicated in mechanisms of entry of virion into cells and egress of virions from cells. Some glycoproteins like gB and gD are essential for viral replication. Other glycoproteins are not essential (gC, gG, gI and gE) for in vitro replication. Other glycoproteins like gD induce strong and consistent cellular immune

response, while gE facilitates cell-to-cell spread of the virus in vivo (**Biewett and Misra, 1991; Chowdhury, 1997; Lyaku et al., 1999; Kevin et al., 1994)**.

Glycoprotein D (gD) is one of the major glycoprotein present in the viral envelop and plasma membrane of virus infected cells **(Van Drunen little-Van Den Hurk etal., 1984; Marshal etal., 1986)**. gD glycoprotein is a type I transmembrane glycoprotein of 417 amino acid encoded by the US6 gene with a calculated molecular weight (MWt) of 71 kDa), with a signal sequence and a cleavage site located between aa 18 and 19 in BoHV-1 gD and this signal sequence is cleaved to yield a mature protein of 399 aa **(Liang., 1995; Dummer et al., 2014).** It stimulates a potent neutralizing antibody response (**Shen et al., 1991; Babiuk et al., 1996)**, thus, gD seems a suitable candidate antigen for the development of immunodiagnostic assays.

Serological detection of antibodies to BoHV-1 is commonly performed using the virus neutralization test (VNT). However, VNT cannot be automated and demands manipulation of infectious virus, requiring cell culture facilities and a great deal of labor, making it time-consuming (3–5 days) and expensive. An alternative to VNT is the use of ELISA for antibody detection, which is designed to be fast, sensitive and relatively inexpensive (**House JA and Baker JA., 1971; Collins et al., 1985a)**. In addition, ELISAs can easily be applied for large scale screening of test samples and allow the use of purified recombinant viral envelope proteins as antigenic substrate; which can be produced in large quantities without handling live viruses and can eliminate the presence of host cellular proteins in the test, reducing false-positive reactions (**Balamurugan et al., 2010; El-Kholy et al., 2013).**

Baculoviruses have been used successfully as vectors for expression of foreign genes both *in vitro* and more recently, in vivo (**Bai et al., 2008; Facciabene et al., 2004)** particularly in the development of pharmaceuticals, gene therapies, diagnostic reagents and vaccines. The traditional method for development of baculovirus expression vectors based on the replacement of a very late, non-essential virus gene coding region with the gene of insert via homologous recombination **(Kost et al., 2005).** Target genes of recombinant baculoviruses were expressed within insect cells undergo most eukaryotic posttranslational modification as in an authentic manner