Polish Journal of Veterinary Sciences Vol. 22, No. 3 (2019), 631–633

DOI 10.24425/pjvs.2019.129971

Short communication

Development of an indirect ELISA based on glycoprotein B gene for detecting of Feline herpesvirus type 1

Y. Tan[1*], G. Dong[3*], J. Niu[1], Y. Guo[1,2], S. Yi[1], M. Sun[1], K. Wang[1], G. Hu[1]

[1] College of Animal Science and Technology, Jilin Agricultural University, Changchun 130118, P.R.China
[2] Animal Husbandry and Veterinary Science Research Institute of Jilin Province, Xian Street No. 4510, Changchun, P. R. China
[3] Beijing Normal University's Global Change and Earth System Science Research Institute, Beijing 100875, P. R. China

Abstract

The study was aimed to develop an indirect enzyme-linked immunosorbent assay (ELISA), which can detect specifically Feline herpesvirus type 1 (FHV-1). The primers were designed based on the conserved sequence of FHV-1 glycoprotein B gene. The recombinant protein with reactogenicity was purified as coating antigen of the assay. The indirect ELISA, characterized by high sensitivity showed no cross-reaction with two types of feline virus, had detection limit at 1:2000 dilution. The positive rate of the assay, according to the determined cutoff value (0.25), was basically consistent with Feline Herpes Virus Antibody ELISA kit. In conclusion, the indirect ELISA with high repeatability and reproducibility can be used for detecting FHV-1, and can provide necessary support to related research.

Key words: feline herpesvirus type 1, gB protein, indirect ELISA, prokaryotic expression

Introduction

Feline herpesvirus type 1 (FHV-1) is a double-stranded DNA virus leading to obvious symptoms of upper respiratory tract infection such as stromal keratitis and chronic rhino sinusitis (Johnson and Maggs 2005), which can make body temperature rise obviously. The virus has the ability of being reactivated under certain conditions to cause the disease recurrence, which was first discovered in the United States, and it can spread by contact and aerosol easily. As previously described, the virus, with mechanisms of immune escape and latent infection, can stay latent in neuronal cells, which makes it difficult to prevent and treat related diseases (Davison et al. 2009). Some study indicated that the glycoprotein B (gB) related to viral replication and infection is the vital neutralizing antigen and vital component of the surface of the FHV-1 capsule (Tikoo et al. 1995), which can mediate the virus binding to heparin-like receptors and plays the role of promo-

Correspondence to: K. Wang, G. Hu, e-mail: guixue1964@126.com
* These authors contributed equally to this work.

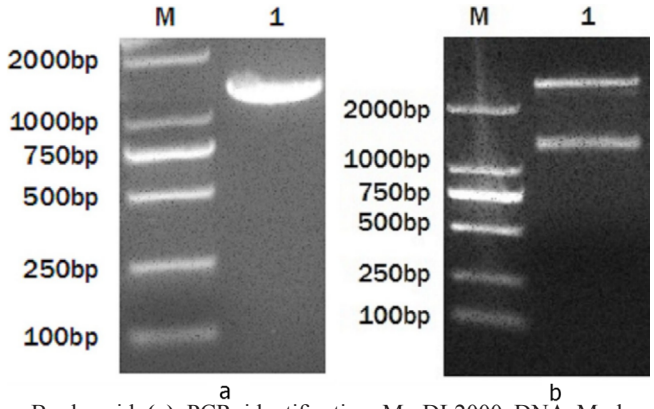

Fig. 1. Identification of pET-28a-gB plasmid **(a)** PCR identification M: DL2000 DNA Marker; lane 1, PCR product (1300bp) of recombinant plasmid. **(b)** Double enzyme digestion (Eco*R* I, *Xho* I). M: DL2000 DNA marker; lane 1, digested fragment.

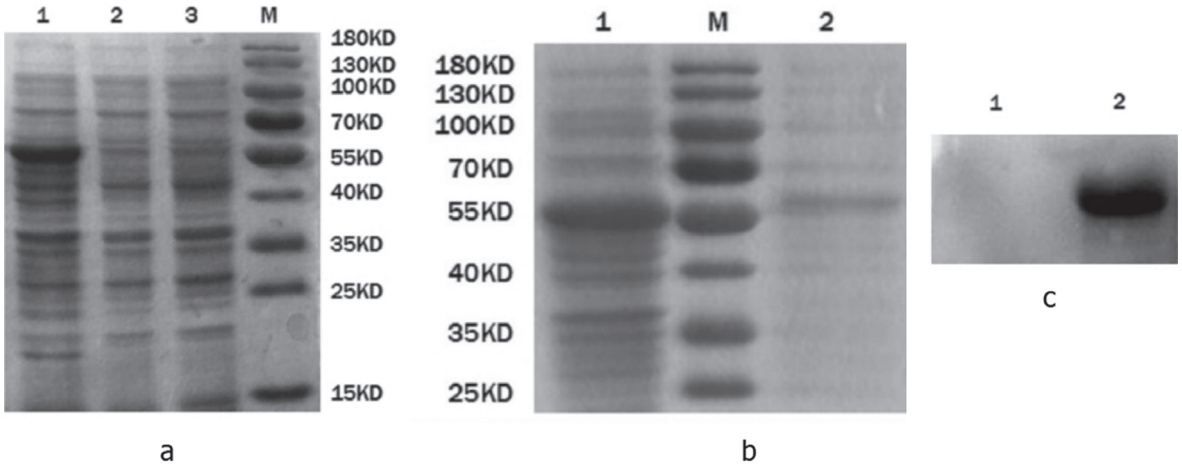

Fig. 2. Identification of gB protein **(a)** Expression identification. M: Protein Marker (15-180 KDa); lane 1, protein expression induced by IPTG (0.4mM, 8 h) (49KDa); lane 2, empty vector control; lane 3, no induced expression control; **(b)** Purification effect. M: Protein Marker (15-180 KDa); lane 1, unpurified protein; lane 2, purified protein (49KDa); **(c)** Western-blot analyses. lane 1, negative control; lane 2, recombinant protein.

ting the fusion between the capsule and cell membranes. Therefore, gB protein can be a coating antigen of detection kits.

The detection methods of FHV-1 include mainly virus isolation, identification and serological reaction. The virus was mainly identified by electron microscopy, regular PCR, fluorescent quantitative PCR, ELISA, as previous research showed (Litster et al. 2015).

Among various diagnostic methods, ELISA can be used on a large scale. The purified recombinant protein can be directly used as a standard antigen in the indirect ELISA, then the assay was developed by optimizing the reaction conditions. His tag was introduced into the terminus of target segment, which can improve the efficiency of protein purification. In conclusion, an indirect ELISA based on gB gene for specific detecting of FHV-1 was developed, which shows sensitivity and has broad application prospects.

Materials and Methods

The primers were designed based on the conserved sequence of FHV-1 gB gene from GenBank (no. S49775.1). Nucleotide sequences of the primers (5'→3') were as follows: gB-F (contain *Eco*R I restriction site), AATTCCCAGTAGAGGGTCAATCTGGAA; gB-R (contain *Xho* I restriction site), CTCGAGGACCGATCTTCTAGTTCGTTGT. The target gene connected to pET-28a (+) (TransGen Biotech, China) was transformed into *E.coli* BL21 Competent Cells (TaKaRa, China). Negative and positive controls were set throughout the assay. The serum samples of 378 cats were collected from Shanghai, Lanzhou and Jilin in China. All samples were collected in accordance with the guidelines and regulations of Animal Care and Use Committee of Jilin Agricultural University, Jilin Province, China.

Results and Discussion

The pET-28a-gB plasmid was built (Fig. 1) and recombinant protein was expressed, the protein was purified (concentration was 328.870 μg/ml) with BugBuster Protein Extraction Reagent (Millipore) and reactogenicity to FHV-1 was proved with Western-Blot identification (Fig. 2). The optimal reaction conditions of ELISA were determined as follows: dilution of recombinant protein – 1:400; dilution of serums – 1:150; protein coating condition – 4°C overnight; type of blocking buffers – 5% BSA; dilution of HRP conjugated Rabbit anti-cat IgG – 1:4000. The cutoff value was determined to be 0.25 according to the formula (average value (0.0855)+3×Standard Deviation (0.054)) that had been reported (Kume et al. 1980), which was based on the OD_{450} value of 65 FHV-1 negative serums.

Sensitivity of the assay was confirmed and the detection limit was at 1:2000 dilution. The assay without cross-reaction with feline calicivirus and feline parvovirus was specific for FHV-1. The coefficient of variation of repeatability and reproducibility were 5.72%, 5.45% respectively, which were all less than 10%. The positive rate of FHV-1 of sample detection was 54.2% (205/378), which was consistent with Feline Herpes Virus Antibody ELISA kit (EVL). In conclusion, the assay with sensitivity and specificity is credible, making a foundation for further development of relevant ELISA kit.

Acknowledgements

This study was financially supported by the National Key Research and Development Program of China (2016YFD0501002), Natural Science Foundation of 13th Five-Year Plan of Jilin Educational Committee (JJKH20190945KJ).

References

Davison AJ, Eberle R, Ehlers B, Hayward GS, McGeoch DJ, Minson AC, Pellett PE, Roizman B, Studdert MJ, Thiry E **(2009)** The order Herpesvirales. Arch Virol 154: 171-177.

Johnson LR, Maggs DJ **(2005)** Feline herpesvirus type-1 transcription is associated with increased nasal cytokine gene transcription in cats. Vet Microbiol 108: 225-233.

Kume K, Sawata A, Nakase Y **(1980)** Relationship between protective activity and antigen structure of Haemophilus paragallinarum serotypes 1 and 2. Am J Vet Res 41: 97-100.

Litster A, Wu CC, Leutenegger CM **(2015)** Detection of feline upper respiratory tract disease pathogens using a commercially available real-time PCR test. Vet J 206: 149-153.

Tikoo SK, Campos M, Popowych YI, Hurk LVD, Babiuk LA **(1995)** Lymphocyte proliferative responses to recombinant bovine herpes virus type 1 (BHV-1) glycoprotein gD (gIV) in immune cattle: identification of a T cell epitope. Viral Immunol 8: 19-25.