Detection of Bovine Herpes Virus-1 in Indonesia by Immunoperoxidase Monolayer Assay

Untari T*,1, Purwandari YK2, Kusumawati A3 and Sutrisno B2

1Department of Microbiology; 2Department of Pathology; 3Department of Reproduction and Obstetric, Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta, Indonesia
*Corresponding author: t_untari@yahoo.com

ABSTRACT

Bovine Herpes Virus-1 cause of infectious balanoposthitis, vulvovaginitis and respiratory diseases. Research had been conducted to develop new technique in BHV-1 diagnostic method. Detection of BHV-1 was conducted using immunoperoxidase monolayer assay (IPMA) with specific monoclonal antibodies anti BHV-1 which were reacted with a secondary antibody labeled horseradish peroxidase. Positive reaction showed in brown color mainly in nucleus of Madin Darby Bovine Kidney cells and the negative showed transparent/colorless cells. The study demonstrate that IPMA was able to detect viral antigen BHV-1 in cell culture.

INTRODUCTION

Bovine Herpes Virus-1 (BHV-1) causes infectious pustular vulvovaginitis, balanoposthitis and respiratory disease. There are only 1 BHV-1 serotype known, which consists of 3 subtype based on the restriction on enzyme cleavage pattern in virus DNA. These subtypes are BHV-1.1 (respiratory subtype), BHV-1.2a, and BHV-1.2b (respiratory and genitalia subtype) (Graham, 2013). The virus neutralization test was regarded as the gold standard (Biswas et al., 2013), based on real time PCR assay for detection BHV-1 in semen and other clinical sample (Chandranak et al., 2013). Pawar et al. (2014) developed of SYBR green based duplex real time PCR for detection of BHV-1 in semen. The disease can be diagnosed by using conventional procedure like Enzyme Linked Immunosorbet Assay (ELISA) (Bishir et al., 2011). ELISA is only to detect the antibody, therefore cannot to differentiate between infection or vaccination. The new technique needs to be developed to detect antigen virus. This research goal’s was to develop Immunoperoxidase monolayer assay (IPMA) to detect BHV-1 antigen using BHV-1 specific monoclonal antibody.

MATERIALS AND METHODS

Samples: Total of 12 nasal swabs were sampled from IBR suspected cattle, from West Sumatera. Samples were collected in Hank’s solution as transport medium and supplemented with MEM (Sigma) medium and Penicillin 500 IU/ml, streptomycin (Thermo Fisher) 100 µg/ml, Fungizone (GIBCO) 2 µg/ml. All of the samples were then centrifuged 3500 rpm for 15 minutes and supernatant were collected, then stored at -20°C until next test.

Cell culture and infection: Madin-Darby Bovine Kidney Cell Line (MDBK) cell culture was used for virus propagation. The medium used media Dulbeco’s eagle Minimal Essential Media (DEMEM) supplemented with 5% fetal bovine serum (Sigma), 1% glutamine, penicillin 1000 IU, 2 mg/ml streptomycin, and fungizone 2 µg/ml. MDBK cell amount 300,000 cell/ml were transferred in 1 ml micro plate flat bottom 24 wells and incubated at 5% CO2 atmosphere for 48 hours. The monolayer was inoculated with 50 µl supernatant of suspensions virus. The plates where then incubated at 37°C in 5% CO2 atmosphere for 48 hour (Silva et al., 2006).

Immunoperoxidase monolayer assay: The method was in accordance to Direksin et al. (2002) with modification. MDBK cells, which had been infected fixated in 50 µl of acetone in PBS that contain 0.02% bovine serum albumin and incubated at room temperature for 10 minutes. Acetone solution were remove, and then incubated at 37°C for 10 minutes. After cell were dried, 50 µl monoclonal antibody anti BHV-1 (1:200) were added to the plate, wich were incubated for 30 minutes at room temperature. After washing with Tween 20, 50 µl streptavidin-horseradish peroxidase (HRP)-labelled was added to all well and the plate were incubated at room temperature for 30 minutes. MDBK cell were washed 2 times before adding 50 µl of Chromogen diamino-benzidine substrate (DAB) for 30 minutes. MDBK cells were washed twice with aquades, dried in oven 37°C for 2 hours, then exemined under a light microscope.

©2016 PVJ. All rights reserved

reacted with streptavidin-horse radish peroxidase. The positive reactions which are characterized by brown staining mainly of the nuclei of BHV-1 infected MDBK cell (Fig. 2A). Negative reaction was characterized by the colourless cells (Fig. 2B). Monoclonal antibody (mo-Ab) are be able to bind with viral protein lead to this reaction more specific than conventional serology test, which cannot differentiate BHV-1 and BHV-5 (Bashir et al., 2011). The IPMA test can be used to detect antigen BHV-1 and not require highly purified antigen. The IPMA is a convenient alternative option for detecting antigen BHV-1 from swab nasal and sperm sample. IPMA was used to detect African swine fever viral protein showed that be highly specific, however polymerase chain reaction is more sensitive than IPMA (Afayoa et al., 2014). ELISA efficiently detect BHV-1 but requires purified antigen (Bashir et al., 2011). BHV-1 detect by real time PCR is less subject to risk of cross contamination than conventional PCR but this method need specific equipment (Crook et al., 2012).

Conclusions: According to the research result, IPMA method can be used to detect BHV-1 infection accurately. This method did not need many requirements such as highly purified antibody. This result should encourage the usage of IPMA as a potential alternative test method in detecting BHV-1 infection.

Author’s contribution: KA had a role to prepare the location of sampling, and taking the sample. While PYK and SB were responsible the pathological examination of immunoperoxidase assay. UT were responsible for performing the assay. The analysis of the result was conducted by all of the authors. All authors approved the final version of the manuscript.

RESULTS AND DISCUSSION

Cytopathic effect in BHV-1 infected MDBK cells was grape like cluster observed after 48 hour post infection (Fig. 1A). IPMA method was developed at cell culture to detect BHV-1 using specific monoclonal antibody. The monoclonal antibodies will bind with antigen BHV-1. The binding can be detected with the addition of secondary antibody-biotinylated, which

![Fig. 1: BHV-1 infected MDBK cell, showed cytopathic effect which grape like cluster (A), while cell control which were not infected appear normal (B).](image)

![Fig. 2: MDBK cell infected with BHV-1, isolate no. 12. Staining with streptavidin-biotin peroxidase method, diaminobenzidine substrate method. Positive infected cell appeared brownish color (A) (400x). Negative MDBK cell, not infected BHV-1 cell appeared transparent/colourless (B) (400x).](image)

REFERENCES


