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SHORT COMMUNICATION

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

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A B S T R A C T

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-1was conducted using immuperoxidase monolayer assay (IPMA) with specific monoclonal antibodies anti BHV-1 which were reacted with a secondary antibody labeled horseradishperoxidase. 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.

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INTRODUCTION

Bovine Herpes Virus-1 (BHV-1) causes infectious pustular vulvovaginitis, balanopostitis 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 (Chandranaik *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 Immunosorbent Assay (ELISA) (Bashir *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 -20ºC 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% CO_2, 37ºC for 48 hour. The monolayer was innoculated with 50 µl supernatan of suspensions virus. The plates were then incubated at 37ºC in 5% CO_2 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 oven 37ºC 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 37ºC for 2 hours, then exemined under a light microscope.

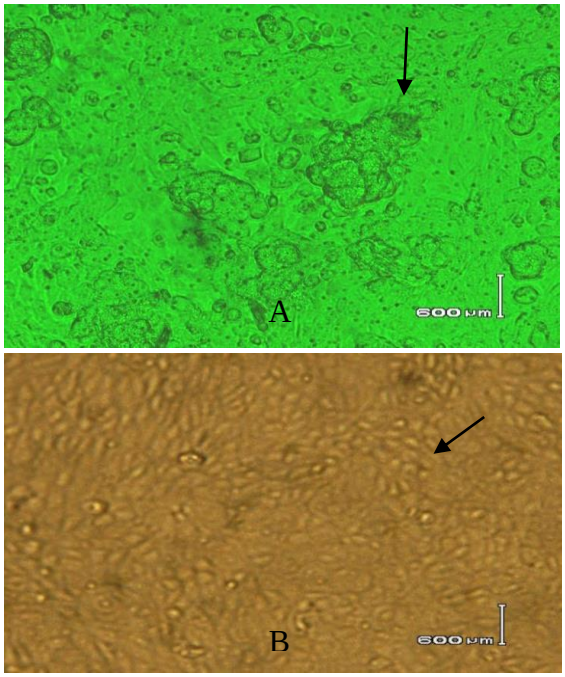

Fig. 1: BHV-1 infected MDBK cell, showed cytophatic effect which grape like cluster (A), while cell control which were not infected eppear normal (B).

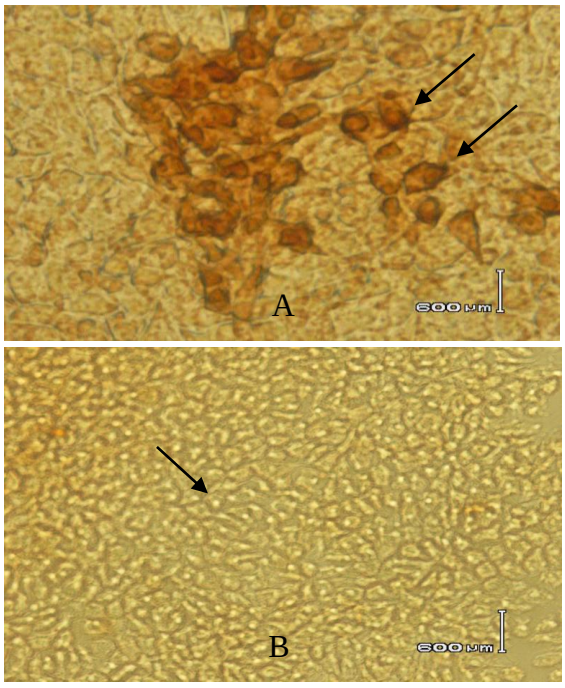

Fig. 2: MDBK cell infected with BHV-1, isolate no. 12. Staining with streptavidin-biotin peroxidase method, diaminobenzidine substrate method. Positive infected cell appeared brownish color (A) (400x). Negative MDBK cell, not infected BHV-1 cell appeared transparent/colourless (B) (400x).

RESULTS AND DISCUSSION

Cytophatic 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 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 spesific, 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 methode need spesific 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.

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