Detection of bovine viral diarrhea virus (BVDV) in milk samples by immunoperoxidase monolayer assay

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Abstract

Bulk milk samples from each individual herd that had histories of reproductive problems were collected to investigate the possibility of adaptation of immunoperoxidase monolayer assay (IPMA) for the detection of Bovine Viral Diarrhea Virus (BVDV) in milk samples. Blood samples from the same herds were used as standard for direct comparison. Using IPMA involving blood and milk samples from 3 out of 5 herds with reproductive problems showed positive results. This indicates the usefulness of milk samples for identifying herds infected with BVDV.

Key words: Bulk milk - reproductive problems - immunoperoxidase monolayer assay - BVDV

Introduction

Bovine Viral Diarrhea Virus (BVDV) is an RNA virus which is classified in the genus Pestivirus in the family Flaviviridae (Westaway et al., 1985). BVDV is worldwide and is one of the most economically important viral pathogens to cattle. Based on cell culture analysis, BVDV is divided into cytopathic (CP) and noncytopathic (NCP) biotypes (Gilje et al., 1960). Unlike the CP-BVDV, the NCP-BVDV can cross the placenta barrier, so if a pregnant cow becomes infected with an NCP-BVDV biotype during first trimester of pregnancy, she can produce calves which will develop immunotolerance toward that NCP-BVDV strain and also become persistently infected with the virus which will spread the virus throughout its lifetime (Roeder et al., 1986). The prevention and the control of BVDV is therefore relied upon the existence of a rapid but accurate screening technique for detection of persistently-infected animals in herds.

Immunoperoxidase monolayer assay (IPMA) is an assay that has been successfully developed to detect persistently-infected animals within seropositive herds using sera as samples (Wasito and Wuryastuti, 1992). The IPMA is relatively inexpensive and is better adapted to processing larger amount of samples (Wasito and Wuryastuti, 1993). Since the BVDV is also present in other body fluids, such as milk, urine and uterine secretions, we therefore, investigated the possibility of adaptation for the detection of BVDV in milk samples which may ease detection herds infected with BVDV.

Materials and methods

Two hundred milliliters of bulk milk samples were collected from individual herds in a sterile containers and held on ice for transport to the laboratory. Five herds were sampled that had histories of abortions and/or other reproductive problems. In addition, 5 herds were sampled that had no history of clinical disease suggestive of BVDV infection. For comparisons, blood samples from the same herds were collected and used as the standard to which the milk samples were directly compared.

Somatic cells were isolated using a modification method of Paape and Miller (1990). The milk samples were aliquoted into 4×50 ml conical tubes and were then defatted by centrifugation at 1,800 rpm for 15 minutes at 4°C. After centrifugation, the supernatant with the fat layer was discarded and the cell pellet was washed twice in cold 0.01 M PBS pH 7.2 by centrifugation at 1,500 rpm for 5 min. The washed cells were then suspended in approximately 250 µl of EMEM. The cells pellet from the 4 conical tubes were transferred into a 1.5 ml eppendorf tube. The tube was microcentrifuged at 5000 rpm, 4°C for 1 min. The supernatant was discarded and the cells pellet was resuspended in 1 ml of EMEM supplemented with 10% of equine sera. The virus in the cells-suspension was collected by three times freezing and thawing followed by removing the cell debris by centrifugation at 2500 rpm, 4°C for 5 min. After centrifugation, the supernatant was collected into a new eppendorf tube for IPMA or frozen at -80°C until processed.

Culturing Bovine Turinate (BT) cells for IPMA was done by dropping 50 µl of BT cells suspension (300,000 cells/ml) into every well of a sterile, flat-bottomed microtiter plate. One hundred microliters of EMEM media supplemented with 10% of equine sera were added into every well containing BT cells suspension and the cells were incubated for 24 hours at 37°C in a 5% CO₂ incubator. After incubation, 25 µl of virus suspension were added to each well. Infected BT cells were harvested 72 hours post infection and fixed using 35% acetic in phosphate buffered saline containing 0.02% bovine serum albumine.

The occurrence of BVDV in cultured BT cells were detected by the IPMA as described by Waseito and Hastari (1992 and 1993). One hundred microliters of diluted swine polyclonal antibody anti-BVDV were added to each well containing the acetone fixed BT cells and were incubated for 30 minutes at room temperature. The microtiteration plate was drained off and the cells were rinsed three times using wash buffer. Fifty microliters of diluted protein G-Horseradish peroxidase were added to each well and incubated for 15 minutes at room temperature. After incubation, the microtiteration plate was again drained off and was rinsed two times with wash buffer. The 100 µl of substrate solution was added to each well and was incubated in the dark at room temperature for 60 minutes. After incubation, the microtiteration plate was drained, rinsed twice with tap water and finally the plate was dried and searched under microscope for the BVDV.

Results

Seventy-two hours after infection of bovine turbinate cells with somatic cells isolated from the bulk milk samples, 3 out of all samples were identified as positive for BVDV as detected by immunoperoxidase staining (Fig. 1). IPMA testing on BT cells infected using blood samples collected from the same herds showed the same results (Table I). This confirmed the presence of BVDV infection in those particular herds.
Discussion

Results showed that the use of immunoperoxidase staining in this study has allowed the detection of BVDV in 3 out of 5 herds which have reproductive problems suggestive of BVDV infection. This indicates that the technique had been successfully adapted for detecting BVDV using bulk milk as samples. The results also proved that application of this technique would enable us to easily screen the presence of the BVDV in a herd.

This is very important especially for the herd that has immunotolerant animals which appears clinically normal but shed BVDV throughout its life (McClurkin et al., 1964).

The sensitivity of IPMA for detecting BVDV using bulk milk samples, however, need to be further determined. This is mainly because factors such as number of infected animals and clinical progression of the infection within the herd and time-course of the herd infection could influence the overall results. Therefore, up to the present, the application of the technique is only useful for identifying BVDV positive herds for control strategies and is not as a definitive assay for ensuring the absence of BVDV infection within the herd.

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References


tase polymerase chain reaction to detect bovine viral diarrhea virus. A 3 month post-doctoral project. Department of Microbiology and Animal Health Diagnostic Laboratory, Michigan State University-Biotechnology Career Fellowships of the Rockefeller Foundation, USA.


Table 1. Results of immunoperoxidase monolayer assay in 10 different herds using blood and bulk milk as samples

<table>
<thead>
<tr>
<th>No.</th>
<th>Herds</th>
<th>History of reproductive Blood</th>
<th>IPMA*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Gp-H</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Se-H</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>PWw-H</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>MSL-H</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Pb-H</td>
<td>+</td>
<td>-</td>
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</tr>
<tr>
<td>10</td>
<td>Uz-H</td>
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*Immunoperoxidase monolayer assay