Organizing Institutions
- Study Center For Bali Cattle, Udayana University, Bali, Indonesia
- Faculty of Veterinary Medicine, Udayana University, Bali, Indonesia
- Faculty of Animal Husbandry, Udayana University, Bali, Indonesia

Steering committee
Prof. Dr.Ir. Nyoman Suparta, MS, MM (Indonesia)
Prof. Dr. drh. I Made Damriyasa, MS (Indonesia)
Prof. Dr. Matthias Gauly (German)
Prof. Dr. Christian Bauer (German)
Dr. Collin Cargil (Australia)

Organising committee
Prof. Ir. I Gusti Lanang Oka, M.Agr., PhD. (Head)
Dr. drh. Ni Ketut Suwiti, MKes. (secretary)
Prof. Dr. Ir.Ida Ayu Made Sukarini, MAgr. (Tresure)

Editors:
Prof. Ir. I DK. Harya Putra, MSc., PhD
Prof. Ir. I Gusti Lanang Oka, M.Agr., PhD
Prof. Dr. drh. I Made Damriyasa, MS.
Prof. Ir. Sayang Yupardi, MSc.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Title</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foreword</td>
<td>iii</td>
</tr>
<tr>
<td>Schedule Program International Seminar</td>
<td>iv</td>
</tr>
<tr>
<td>Table of Contents</td>
<td>ix</td>
</tr>
<tr>
<td>Stunning, Slaughtering And Beef Carcass Classification</td>
<td>1</td>
</tr>
<tr>
<td>E. Tonges</td>
<td></td>
</tr>
<tr>
<td>Reproduction Management And Breeding Strategies To Improve Productivity And Quality Of Cattle</td>
<td>2</td>
</tr>
<tr>
<td>Kusuma Diwyanto and Lisa Praharani</td>
<td></td>
</tr>
<tr>
<td>Bovine Neosporosis (<em>Neospora Caninum Infection</em>) In Indonesia And Other Southeast Asian Countries</td>
<td>3</td>
</tr>
<tr>
<td>Christian Bauer</td>
<td></td>
</tr>
<tr>
<td>Functional Traits In Cattle Breeding, Is There A Potential For Bali Cattle?</td>
<td>4</td>
</tr>
<tr>
<td>M. Gauy</td>
<td></td>
</tr>
<tr>
<td>Strategic Role of Micro Finance Institution/Unit (MFIOr MFU) on Supporting Beef Self Sufficiency: A Concept</td>
<td>5</td>
</tr>
<tr>
<td>Ashari, E. Juarini</td>
<td></td>
</tr>
<tr>
<td>Histopathological Changes of The Liver, Kidney, and Spleen of Bali Cattle That Were Given 2% Concentrate In Grass Feed Berata IK, Kardena IM, Winaya IBO, Ariana INT</td>
<td>17</td>
</tr>
<tr>
<td>Luh Gde Sri Asiti and Tanda Panjaitan</td>
<td>24</td>
</tr>
<tr>
<td>Leaves and Skin Of Pineapples To Control Internal Parasite on Bali Cattle in Lombok</td>
<td></td>
</tr>
<tr>
<td>Histological Studies of Lymph Node in Bali Cattle</td>
<td>28</td>
</tr>
<tr>
<td>Suwiti, NK, Eka Setiasih, NL, Puja, K. Suastika, P. Piraksa, I.W.</td>
<td></td>
</tr>
<tr>
<td>Diagnosis of Jembrana Disease Virus Subunit <em>Env-Tm</em> Gene From Infected Bali Cattle in Kalimantan By Dot-Blot Hybridization</td>
<td>36</td>
</tr>
<tr>
<td>Asmarani Kusumawati, Penny Humaidah Hamid</td>
<td></td>
</tr>
<tr>
<td>Financial Analysis of Bali Cattle Yard Given Biofermentation Ration on Inconventional By Product Basis</td>
<td>44</td>
</tr>
<tr>
<td>Putri, B. R. T., Tjok I. Putri, T. G. B. Yadnya, I. M. Mudita, Ni W. Siti, dan J W. Sukanata</td>
<td></td>
</tr>
</tbody>
</table>
DIAGNOSIS OF JEMBRANA DISEASE VIRUS SUBUNIT ENV-TM GENE FROM INFECTED BALI CATTLE IN KALIMANTAN BY DOT-BLOT HYBRIDIZATION

Asmarani Kusumawati1,2, Penny Humaidah Hamid1

1 Faculty of Veterinary Medicine, University Gadjah Mada, Yogyakarta, Indonesia
2 Study Center of Biotechnology, University Gadjah Mada, Yogyakarta, Indonesia

ABSTRACT

Jembrana disease, an acute disease of Bali cattle (Bos javanicus) is caused by Jembrana Disease Virus (JDV), a bovine lentivirus. The availability of specific and rapid detection methods is essential for monitoring the health status of farmed species, particularly in viral disease as in this case early diagnosis is a critical factor in containing disease outbreaks. The possible use of subunit env-tm of Jembrana Disease Virus gene as hybridization probes was tested as Jembrana disease detection tool. This gene has been cloned in PGEX-2T and the probes, 211 bp and 252 bp long, were prepared by PCR and Dig-labeled. Dot-blot hybridization was carried out on total RNA extracted from blood of Bali cattle suspected Jembrana disease. Result showed that the method allowed the detection of JDV originating from Kalimantan. It allowed the detection of the virus in total RNA as low as 10 pg. Due to its ease of use, it is a good method in conditions where sophisticated equipments are not available.

Key words: dot-blot hybridization, env-tm gene, Bali cattle, Jembrana Disease Virus

INTRODUCTION

Jembrana disease virus (JDV), a newly recognised bovine lentivirus detected in Indonesia (Chadwick et al., 1995), causes an acute, severe disease in Bali cattle (Bos javanicus) and a mild disease syndrome in Bos taurus. Infection of Bali cattle with JDV results in a case fatality rate of approximately 20%; the remainder survive with no recurrence of disease (Soesanto et al., 1990). The genome of JDV is composed of two linear single stranded RNA viruses. The detection of single-stranded RNA is prone to degradation until recently the RT and
the amplification reaction could be performed in one step. In the absence of any genome sequence data, a sandwich enzyme linked immunosorbent assay (S-ELISA) was developed using antibodies produced in mice. The drawbacks of the methods is the limited stocks of antibodies, unless they are produced by hybridoma technology and its possibility of bovine immunodeficiency virus (BIV) cross reaction, the other similar virus.

This study aims to produce more reliable and sensitive detection methods based on nucleic acid. We used the conserved env-tm sequences from Bali samples to design a high specific probe based on its stable genetic content. In this paper we describe the employment of digoxigenin-labeled probes for Jembrana Disease Virus detection. We developed a dot-blot hybridization based on env-tm subunit gene, an easy and suitable method for monitoring health status and epidemiology studies.

**MATERIALS AND METHODS**

**Specimens examined**

Whole blood was collected in 10-ml EDTA Vacutainer tubes (Becton Dickinson Vacutainer System) from suspected Bali Cattle in Barito Kalimantan. Samples were stored as 1-ml aliquots at -80°C until they were required.

**Extraction of viral RNA.**

RNA extractions were carried out with 200 µl whole blood samples by using Viral RNA Isolation Kit (Roche Applied Science), according to the manufacturer's instructions.

**Plasmid preparation**

Previous cloning of env-tm gene in pGEX-2T (Amersham Pharmacia) was PGEX-TM construct which was available in our laboratory and kept frozen in Luria Bertani medium containing glycerol. Plasmid were extracted using High Pure Plasmid Isolation Kit (Roche Applied Science) according to the manufacturer’s instruction. Plasmid were analysed by PCR amplification using upstream primer ATAGGATCCATGGCGGTGGGATGGTCATAT and downstream primer CAGCGGATCCTGCAAGCTACGTGTC for detection of env-tm insert.
Probe preparation

Probes were synthesized using upstream primer AGAAGCTCAGCGAAGGCA and downstream primer TTTCTCCCCACAGTCCAC for JT1. Second probe (JT2) used upstream primer ATCTGAACACACGAGGTTAATAATC and downstream primer CTAAGGCTCAGACCAATTG. Plasmid PCR was carried out in 50 µl reaction containin 5 ng pGEX-TM, PCR DIG labelling mix (200 µM dNTP, digoxigenine-11-dUTP) (1 ul), 1-5 unit Taq DNA polymerase (1 ul), and 50 pmol every primer of the probes.

Dot-blot hybridization

Hybridization was carried out using RNA obtained from blood. Samples were denatured in MOPS buffer (2 mM MOPS [3-N-morpholino-2-hydroxpropane sulfonic acid], 5 mM sodium acetate, 1 mM EDTA, pH 7.0), 50% formamide and 2 M formaldehyde, at 65°C for 5 min, and then chilled in ice. One µl of each sample was then dot-bloated in duplicate on Hybond-N membrane (Amersham-Pharmacia). The membrane was dried at 50°C for 1 h, then cross-linked under UV for 3 min. Pre-hybridization was carried out at 42°C for 3 to 4 h in 50% formamide, 2 × saline sodium citrate (SSC), 50 mM sodium phosphate, 2% blocking reagent, 0.1% sodium sarkosyl, and 7% sodium dodecyl sulphate (SDS). Hybridization was performed overnight at 42°C by adding 5 to 25 ng ml⁻¹ of denatured labeled probe. The membrane was washed twice with 2 × SSC, with 0.1% SDS for 15 min at room temperature, then twice with 0.5 × SSC, with 0.1% SDS for 15 min at 68°C. Detection was done as follows, and all the successive steps were carried out at room temperature: the membrane was first washed in Washing Buffer I (100 mM maleic acid, 150 mM NaCl, pH 7.5, 0.3% Tween 20) for 1 to 3 min, blocked with blocking solution (100 mM maleic acid, 150 mM NaCl, pH 7.5, 1% blocking reagent) for 1 h, then washed twice with Washing Buffer I for 15 min, and finally washed once more with Washing Buffer II (100 mM Tris-HCl, pH 9.5, 100 mM NaCl, 50 mM MgCl²) for 5 min. The presence of hybridized probes was revealed by incubating the membrane in 2 ml of Buffer II, containing 9 µl nitroblue tetrazolium (NBT) and 7 µl X-phosphate solution, in a dark room for a minimum of 2 h or overnight.

Image analysis
Result were scanned and densitometry measured using the program "NIH Image 1.60 for densitometric analysis".

RESULT AND DISCUSSION

Figure 1. A. shows isolation of *env-tm* gene from *E coli* DH5α. Previous cloning of *env-tm* gene in pGEX-2T allowed us to isolate *env-tm* gene easily and rapidly. It confirmed that clone pGEX-TM gave a fragment of about 6.0 kb, consist of 4.9 kb pGEX-2T and 1.1 kb *env-tm* gene. *env-tm* gene insertion analysis was also done by PCR method using specific primer to amplify *env-tm* fragment. PCR analysis gave a specific fragment of around 1.1 kb showed by figure 1. B. The result showed us that *env-tm* was exist. This result also showed that insert can be analysis by PCR method using specific primer.

![Figure 1](image)

Figure 1. (A). pGEX-TM 6.0 kb DNA plasmid recombinant electrophoresis. (B). *env-tm* gene PCR from linearized plasmid. Marker was indicated

Sequencing analysis showed point mutation on 619-621 compared to Tabanan 1987 (Chadwick *et al.*, 1995). CAA (619-621 position) mutated to CAG, both of codon are glutamin genetic code (amino acid number 207). It can be happened because of viral genomic evolution or genetic variation on Tabanan 1995 isolate had been used. Error due to sequencing had a minimum value because sequencing result had same data with other sequencing result obtained from pcDNA-TM clone that inserted independently (Kusumawati *et al.*, in press). Sequence analysis was conducted with BLAST programs (Altschul *et al.*, 1997) from NCBI showed *env-tm* gene
inserted in pGEX-TM had high homology identity of around 90% up to 100%, E value 0.0, perfectly

![Sequence Diagram](image)

**Figure 2.** Location of JT1 and JT2 probes within env-tm gene.

Sequence comparison showed that nucleotide sequence of JT1 and JT2 specific to env-tm sequence exhibited high homology identity (97%) of several isolates of JDV originating from several areas in Indonesia. JT1 probes located between 7190 and 7400 of JDV genome (211 base pair). JT2 probes located between 6638 and 6889 of JDV genome (252 base pair). Due to its high conservations, the probes are suitable for detection of JDV in dot blot hybridization and allow to detect all JDV isolates in Indonesia. By applying the probe created in this study to BLAST program, we found no matches through all the sequences currently listed in GenBank except for each of the corresponding isolates of JDV.

The sensitivity of the hybridization was evaluated by spotting decreasing amounts (1 ng-10 pg) of total RNA on nylon membranes. As shown in Fig. 3 and Fig. 4, the positive response showed up to 10 pg of total RNA.
Figure 3. Dot-blot hybridization using JT1 Dig-labeled probes using total RNA extracted from blood, with 1 ng (1), 100 pg (2) and 10 pg (3) of the target. Positive control, pGEX-TM (I), various samples from Tabanan (II-IV), various samples from Kalimantan (V-XIII), negative control (XIV)

Figure 4. Dot-blot hybridization using JT2 Dig-labeled probes using total RNA extracted from blood, with 1 ng (1), 100 pg (2) and 10 pg (3) of the target. Positive control, pGEX-TM (I), various samples from Tabanan (II-IV), various samples from Kalimantan (V-XIII), negative control (XIV)
Dot blot hybridization with digoxigenin-labeled probes has several advantages for screening clinical samples for JDV virus. The method is relatively fast, being completed in 2 days. It is also relatively inexpensive, mainly because of the long shelf life of the probe. In practice, we reused hybridization mixtures 10 or 12 times before they had to be replaced. This meant that, since two filters with approximately 90 samples each were hybridized together (back to back), up to 2,000 samples could be examined with each probe preparation. In our laboratory, therefore, new probe would have to be prepared only three times per year for sufficient reagent to screen routine samples.

The safety advantages of nonradioactive systems are well known, and it was convenient not to be obliged to do hybridization assays in areas of the laboratory set aside for radioactive work. The nonradioactive dot blot hybridization assay described here provided a safe, economic, and relatively fast screening test for JDV virus and could be reliably used. Its sensitivity was greater than that of other assays routinely available in our laboratory for the detection of JDV virus and was adequate for diagnostic work, giving an increased yield of virus-positive samples.

ACKNOWLEDGEMENTS

This work was funded by Hibah Kompetisi, Directorate General of Higher Education, Ministry of National Education, Republic of Indonesia

REFERENCES


Chadwick B.J., Desport M., Brownlie J., Wilcox G.E., Dharma D.M. 1998. Detection of Jembrana disease virus in spleen, lymph nodes, bone marrow and other tissues by in situ hybridization of