PROCEEDING

INTERNATIONAL SEMINAR

ADVANCED TECHNOLOGY ON VETERINARY AND LIFE SCIENCE

NO. ISBN: 978-979-96104-4-7

YOGYAKARTA
MARCH 12nd, 2011

FACULTY OF VETERINARY MEDICINE
UNIVERSITAS GADJAH MADA

ISBN: 978-979-96104-4-7
Title Proceedings on International Seminar Advanced Technology on Veterinary and Life Science
Published By Faculty of Veterinary Medicine Universitas Gadjah Mada
Address
Jalan Fauna 2 Karang Malang Yogyakarta 55281
Phone: +62-274-560860, 560865 Fax: +62-274-580861

March, 2011

List of Thematic Oral Presentation

Topic 1 : Basic Science
Sub Topic: Molecular Biology

1. The Possible Use of Sub Unit GAG-CA Gene (JC1) As Hybrization Probe Candidate
   Bambang Sutrisno, Penny Humaidah H., Asmarani Kusumawati, Hastari Wuryastuti and J. Sriwidada

2. Development of ELISA system using specific antigen LipL32 and LigA as effective diagnose tools for leptospirosis
   Dyah Ayu Widiasih, R. Isozumi, K. Yoshimatsu dan J. Arikawa

3. Molecular characteristic of Nucleoprotein gene of Rabies Virus isolated from Bali Indonesia
   Heru Susetya dan Nyoman Dibia

4. Detection of Jembrana Virus Using RT-PCR
   Narendra Yoga Hendarta and Asmarani Kusumawati

5. Molecular Epidemiology of Verecytotoxigenic Escherichia coli (VTEC) in Livestocks in Yogyakarta
   Yatri Drastini, Setyawan Budiharta, Widya Asmara, Hastary Wuryastuti dan Langkah Sembiring

Topic 2 : Basic Science
Sub Topic: Applied Science

1. Development of Catecholaminergic in the ventral mesenchepalon of longtailed macaque (Macaca fasicularis) during prenatal Period
   Tri Wahyu Pangestiningsih, Ariana, Djoko Pamungkas and Erni Sulistyawati

2. Pare Extarct (Momordica charantia L) as an Antioxidant Potency For Reducing Blood Glucose Level on Pre-Diabetic Rat (Spague dawley) Model Stability of Sardine Fish Oil and Hydrolyzed Blood Protected to Increase Productivity
Erni Sulistiawati, R.P. Agus Lelana, and Tri Wahyu Pangestiningsih

3. Acute Toxicity Of Cashew Nuts Shell Extract (Anacardium occidentale L) in Albi Rat: A Preliminary Study
Harlita, Nur Handayani, NS., Sagi, M., Astuti, P.

Topic 3 : Basic Science
Sub Topic: Applied Science

1. Mesenchimal stem cell: Potensial Drug For Regeneratif Medicine
Yudha Heru Fibrianto

2. Concentration of Estradiol in Nilem (*Osteocilus hasselti C.V.*) under Photoperiods Manipulation
Prayogo, N.A., G.E. Wijayanti, Murwantoko san P.Astuti

3. Rapid Diagnosis of Avian Influenza Virus Subtype H5N1 by using Single Step Multiplex RT-PCR method
Aris Haryanto, Sri Handayani I. dan Dini Wahyu Y.

Topic 4 : Basic Science
Sub Topic: Basic Anatomy

1. Comparison Distribution of Various Lectins on The Stomach of the Java Fruit Bat (Rosettus sp.) and The Java Insect Bat (Myosit sp.)
Ariana and Teguh Budipitojo

2. Immunohistochemical Study of The Ghrelin In The Stomach of Java Porcupne (Hystrix Javanica)
Teguh Budipitojo, Motoki Sasaki, Ariana, Soehartini Jatman, and Nobuo Kitamura

Topic 6 : Preclinical Science
Sub Topic: Fishery

1. Identification Species of Myxobolus From Gill of Cyprinus Carpio in East Java
   Agus Priyono, Kurniasih, Rini Widayanti, Ayuda D. Nurekawati

2. Early In Vitro Oogenesis Of The Giant Gouramy (*Osphronemus gouramy Lac*)
   Aulidya Nurul Habibah, Isdy Sulistyo, and Gratiana E. Wijayanti

3. Identification of Clinostomum complanatum (Digenea: Clinostomidae) in Freashwater Fish in Yogyakarta and Riau, Indonesia Based on Molecular Study Fish in Yogyakarta and Riau, Indonesia Based on Molecular Study
   Morina Riauwaty, Kurniasih, Joko Prastowo and Windarti

4. Histopathological Changes of Edwardsiellosis in Cyprinus carpio From Pontianak, West Kalimantan, Indonesia
   Siti Narwiyani, Kurniasih and Asmarani Kusumawati

5. Pathogenecitic differences of Aeromonas salmonicida isolates collected from fresh water fish in four areas of Indonesia
   Riza Priyatna, Kurniasih, S. Indarjulianto and Surya Amanu

Topic 7 : Clinical Science
Sub Topic: Large Animal and Poultry

1. Effects of Varying Egg Yolk Addition On Post-Thawing Sperm Function of Ettawa Crossbreed Goats
   Erif Maha Nugraha Setyawan

2. The Effect Addition of Red Ginger Phytobiotic to The Production and Blood Profile of Broiler Chicken
   Herawati

3. Phosphate Homeostasis In Pre-Ruminant And Young Ruminant Goats
   Irkham Widiyono

4. Profile Of Some Blood Chemistry Parameters In Crossbred Ettawa Goats
Irkham Widiyono and Sarmin

Topic 8 : Clinical Science
Sub Topic: Reproduction and Large Animal

1. Stability of Sardine Fish Oil and Hydrolyzed Blood Protected to Increase Productivity of Dairy Cattle
Ahmad Pramono, Kustono, Prabowo P.P., Dyah Triwidayati and Hari Hartadi

2. Folicular Dynamic On Repeat Breeding Crosbreed Cows Examined Using Ultrasonography
Surya Agus Prihatna, Prabowo P.P., Sri Gustari

3. Physiological Functions of Betaine in Monogastric Animals
Adi Ratriyanto

4. Nuclear Maturation Of Immature Porcine Oocytes After In VitroMaturation Following Vitrication
Agung Budiyanto

DETECTION OF JEMBRANA VIRUS USING RT-PCR

Narendra Yoga Hendarta[1,2] and Asmarani Kusumawati[2,3]

1. Health Analyst Department, Yogyakarta Health Polytechnics, Yogyakarta, Indonesia
2. Faculty of Veterinary Medicine, University Gadjah Mada, Yogyakarta, Indonesia
3. Study Center of Biotechnology, University Gadjah Mada, Yogyakarta, Indonesia

Corresponding author at : Health Analyst Department, Yogyakarta Health Polytechnics, Yogyakarta, Indonesia; e-mail : narendrayogahendarta@yahoo.com

Introduction

Jembrana virus (JV) is a bovine lentivirus genetically similar to bovine immunodeficiency virus(Chadwick et al., 1995) that causes an acute, severe disease syndrome in infected Bali cattle *(Bos javanicus)* in Indonesia with a mortality of about 17% (Soesanto et al., 1990). The acute disease associated with JV infection has a short incubation period of 5-12 days and a duration of about 7 days (Soeharsono et al., 1990). The infection is characterized by fever, lethargy, anorexia and enlargment of superfisial lymph nod (Soesanto *et al.,* 1990) which in recovered animal the virus is still detectable in blood for 2 years after infection (Dharma et al., 1991). Although JV infect Ongole cattle (*Bos indicus*), Fresian cattle (*Bos taurus*) and buffaloes (*Bubalus bubalus*), the clinical sign are milder and the infectious agent only persist for maximum of 9 months in buffaloes or shorter period in other species (Soeharsono et al., 1995). Diagnosis of Jembrana disease is an constitute important to control disease and avoid serious economic losses in farming. Various diagnostic methods based on clinical sign and antigen-antibody reaction has been as routine procedure but has disadvantage because the clinical sign could similar to other illness and antibody to JV is not detected in cattle until

after recovery from the acute disease, and is not seen in a majority of cattle until 11 weeks after infection (Hartaningsih et al., 1994). Molecular techniques based on genomic sequence such as revers transcription PCR are sensitive, accurate and rapid. Recent this technique has been a usual standard in laboratory practice especially due to portable devices designed for small laboratory.

Material and Methods

2.1.Isolation of total RNAs

Total RNAs were isolated tissues of infected animal using TRIzol reagent (Life Technologies), according to the manufacturer recommandation. Briefly, approximately 70 mg of tissue were homogenized in 1 mL of TriZol then let for 10 min. at room temperature. After centrifugation at 12,000 rpm, for 10 min. in a bench minicentrifuge, the supernatant was treated with 0.2 mL chloroforme then precipitated with 0.5 mL isopropanol. The pellet was washed with 70% ethanol and dissolved in 100 µL of steril DEPC-treated water. The purity and concentration of the RNA preparation were spectrophotometrically established at 260 and 280 nm.

2.2. Recombinat plasmid preparation

For the experiment , recombinant plasmid,pGEX-CA, contain *gag-ca* gen along 670 bp has constructed from research before was used as template. This plasmid is isolated and purified from E. Coli DH5α using GeneJET™ Plasmid Miniprep Kits (Fermentas), followed by spectrophotometric analysis at 260 and 280 nm to determine purity and concentration.

2.3. PCR reaction

A set of two primer, forward (CCAAGAATGCAGAGACACT) and backward (GGCAGTCCTCATTTGCATG) was designed to amplify 232 bp in *gag-ca* gen region. PCR reaction using Dream Green PCR Mix reagent (Fermentas) and pGEX-CA plasmid (10 ng/µl) was conducted to obtain annealing temperature and optimized cycle number. Negatif control were used water and pGEX-2T plasmid as template for each reaction to monitor cross-contamination. In order to test sensifivity, serial dilution of pGEX-CA plasmid (10^{-1} to 10^{-9}) were used as template (original concentration sampel was 10 ng/µl).

2.4. RT-PCR reaction

The obtained of optimized PCR conditions were used to RT-PCR reaction. As RT-PCR reaction, we used previously reverse transcrips reaction and followed amplification of cDNA using various infected tissues as template. This reaction was done in one tube reaction using One Step RT-PCR kit (Roche).

Result and Discussion

3.1. PCR reaction

The optimized PCR reaction was carried out in a final volume of 25 µL, using Dream Green PCR Mix reagent (Fermentas) with the following conditions : firts denaturation at 94°C for 4 min, denaturation at 94°C for 45 sec, annealing at 56°C for 30 sec, elongation at 72°C for 45 sec and additional elongation at 72°C for 10 min. The a set of primer was designed to amplify *gag-ca* gen , a conserve (Desport *et.al*, 2007) structural gen of JV that encode capsid protein virus (Chadwick *et.al.*,1995).The sensivity PCR was performed under the same conditions

which it was able to detect template at 10^{-5} dilution DNA plasmid sample. This result showed the efficiency of PCR slightly high, which based on jurnal report the spesifity of PCR averagely fall at 10^{-2} to 10^{-6}. The PCR efficieny was achieved by beside accuracy of optimized reaction conditions also by primer designing.

3.2. RT- PCR reaction

The following RT-PCR conditions (final volume was 25 µL), first cDNA synthesis at 45^{0}C for 30 min, AMV inactivation 94^{0}C for 2 min, cDNA amplification 40 cycles using PCR conditions discribed previously. RNA virus that extracted from infected tissues such as lymph node, whole blood, meat, and brain was used as template for RT-PCR reaction. The PCR method could detect existency of virus that showed a clear band in 1,8 % agarose with etidium bromide staining after electrophoresis. Different from DNA plasmid as template ,using RNA total usually has lower sensitivity for RT-PCR to detect the viral particles in organs or tissues that may arise from the intricate population of genes and sequences within the total RNA extracted from infected organs or tissues which comprises virus as well as host-cell RNA. But the RT-PCR method was still well detecting the virus.

Conlusions

The RT-PCR method could detect RNA virus from infected tissues and should be value in high sensitivity, rapid, and accurate than conventional detection by clinical sign and antigen-antibody reaction to constitute diagnosis in preventing spread this severe virus. It appropriate for field application in Veterinary Medicine due to portable devices designed for small laboratory.

Acknowledgment

This research was partly financed by the Fundamental Research Grant from Gadjah Mada University, and supported French Embassy in Indonesia .

References

Chadwick BJ, Coelen RJ, Wilcox GE, Sammels LM and Kertayadnya (1995). Nucleotide sequence analysis of Jembrana disease virus: a bovine lentivirus associated with an acute disease syndrome. *J. Gen Virol.*, 76: 1637-1650.

Dharma, D.M.N., Budiantono, A., Campbell, R.S.F., Wilcox, G.E., 1991. Studies of experimental Jembranadisease in Bali cattle. III. Pathology. J. Comp. Path. 105, 397-414.

Desport M., Meredith E. Stewart, Andrew S. Mikosza, Carol A. Sheridan, Shane E. Peterson, Olivier Chavand, Nining Hartaningsih and Graham E. Wilcox . 2007. Sequence analysis of Jembrana disease virus strains reveals a genetically stable lentivirus. *Journal of Clinical Microbiology*

Hartaningsih N, Wilcox GE, Kertayadnya G and Astawa M (1994). Antibody response to Jembrana disease virus in Bali cattle. *Vet. Microbiol.*, 39 : 15-23.

Soesanto M, Soeharsono S, Budiantoro A, Sulistyana K, Tenaya M and Wilcox GE (1990). Studies on experimental Jembrana disease in Bali cattle. II. Clinical signs and haematological changes. *J. Comp. Pathol.*, 103 : 61-71

Soeharsono S, Wilcox GE, Dharma DM, Hartaningsih N, Kertayadnya G and Budiantono A (1995). Species differences in the reaction of cattle to Jembrana disease virus infection. *J. Comp. Pathol.*, 112 : 391-402

Wilcox GE, Chadwick BJ and Kertayadnya G (1995). Recent advances in the understanding of Jembrana disease. Vet. Microbiol., 46 : 249-255.