

Proceedings of the
International Conference on
**ANIMAL & HEALTH
HUMAN & SAFETY**

PROCEEDINGS



- 6 - 8 December 2009
- Palm Garden IOI Resort
Putrajaya, Malaysia

Editors

M. Ariff Omar • Rasedee Abdullah
Gurmeet Kaur Dhaliwal
Chen Hui Cheng • M. Murugaiyah
Kalthum Hashim • Ooi Peck Toung



Universiti
Putra
Malaysia



Universitas
Airlangga
Indonesia



Department of
Veterinary Services
Malaysia

Content

Preface

Plenary Papers

1. Meeting the Challenges of Animal and Human Health Together in Malaysia
Mohammad Azmie Zakaria 1
2. Challenges of Dairy Cattle Development in Indonesia
I Wayan Teguh Wibawan and R. Kurnia Achjadi 2
3. Crossing Boundaries: Wildlife and Humans
Pornchai Sanyathitiseree 5
4. Practicing Green Farming and Waste Management Through Community Services for Veterinary Students
Bambang Sumiarto and Aris Purwantoro 8
5. Control of Zoonotic Diseases in Myanmar
Aung Tun Khaing 9
6. One World One Health - The Only Way to Address Animal Health and Human Safety Concerns
Muhammad Nawaz 18
7. H5N1 Vaccination in Poultry: Background and Update
Abdul Rahman Omar 19
8. Healthy Animals, Safe Foods and Healthy Man
Saleha Abdul Aziz 24

Research Papers

1. Identification of Gastrointestinal Worms from Egg stool Samples of Sambar Deer (*Cervus unicolor*) in Surabaya Zoological Garden
S Koesdarto, P Kusumaningtyas and IS Yudaniayanti 25
2. Daily Dynamic Population of Tabanid Fly in the Teaching Farm of Faculty of Veterinary Medicine, Airlangga University, Surabaya, Indonesia
R Sasmita 30

3.	Detection of <i>Trypanosoma evansi</i> in Horses in West Malaysia Using Conventional Parasitology Techniques and Polymerase Chain Reaction <i>El Elshafie, RA Sani, A Bashir, L Hassan and RSK Sharma</i>	34
4.	Strategies to Combat Anthelmintic Resistance in Small Ruminants <i>C Panchadcharam, N Raimy, Z Che Mamat, P Bathmanaban, Z Zahari, IL Victo, J Omar and A Musbah</i>	38
5.	The Detection of Influenza Viruses in Live Bird Markets in Surabaya City, Indonesia <i>ATS Estoepangestia and AP Rahardjo</i>	42
6.	Hemagglutinin Reactivity of Several Avian Influenza H5-isolates to Vaccinated Chicken Sera <i>A. P. Rahardjo and ATS Estoepangestia</i>	46
7.	Moulting as a Problem in Poultry and How to Solve It <i>E Safitri and M Hariadi</i>	50
8.	Characterisation of Protein Antigen of <i>Streptococcus agalactiae</i> as Causative Agent of Subclinical Mastitis in Dairy Cattle as Vaccine Candidate <i>AETH Wahyunii and WT Wibawan</i>	51
9.	The Ability of Primer IS900 and F57 to Detect <i>Mycobacterium avium</i> Subspecies Paratuberculosis by Conventional PCR <i>WS Nugroho, M. Sudarwanto, DW. Lukman, S Setyaningsih, A Ahmed Hassan and E Usleber</i>	55
10.	Detection of Vancomycin-Resistance Enterococci in Chickens and Workers from Selected Export Farms in Johor, Malaysia <i>YM Getachew, L Hassan, Z Zakaria, AA Saleha and MZ Che Zalina</i>	57
11.	The Efficacy of Recombinant VP2 Infectious Bursal Disease Local Isolate as a Candidate Vaccine <i>R Ernawati</i>	61
12.	Molecular Characterization of Genome Coding for Nucleoprotein and Glycoprotein Rabies Virus from Several Geographical Areas in Indonesia <i>Suwarno</i>	65
13.	Detection of Pathogenic Leptospire Isolates from Water and Soil Samples <i>F Ridzlan A. Rashid, AR Bahaman, S Khairani-Bejo and AR Mutalib</i>	70

14. Occurrence and Virulence Gene Of Non-O157 Shiga Toxin-Producing <i>Escherichia coli</i> from Goats in Selangor, Malaysia <i>AC Tay Zar, AA Saleha AR Mutalib, M Murugaiyah and Z Zunita</i>	72
15. Loop-mediated Isothermal Amplification (LAMP) as a Diagnostic Tool of Jembrana Disease Virus <i>A Kusumawati, NY Hendarta, S Hartati, P Astuti and T Untari</i>	75
16. Wild-type Rabies Virus Glycoprotein to Make Monoclonal Antibody for Early Detection with DAS-ELISA <i>J Rahmahani, Suwarno, SS Andayani and Kusnoto</i>	76
17. <i>In Vitro</i> Digestion of Palm Kernel Cake Fermented with <i>Aspergillus niger</i> and <i>Rhizopus oryzae</i> <i>M Ramin, AR Alimon and M Ivan</i>	80
18. Comparison of the Effect of Kibbled Carob Pods and Barley as Energy Sources at Various Straw: Concentrate Ratios with Untreated or Ammoniated Wheat Straw on the Rate and Extent of <i>In Vitro</i> Fermentation of Straw Based Diets <i>IS Milad, C Rymer and RW Radley</i>	83
19. Effect of Methionine and Threonine Supplementations on Performance of Broiler Chickens Challenged With Oral Infectious Bursal Disease Virus Vaccine <i>E Moroufyan, A Kasim, SR Hashemi, AR Soleimani, TC Loh and M Hair-Bejo</i>	95
20. Effects of Preconditioning and Extrusion of Linseed on the Rumen Fermentation of Dry Holstein Cows <i>F Akraim, M-C Nicot and F Enjalbert</i>	98
21. Morphological Changes of the Intestine in Broiler Chickens Fed Herbal Plants and Acidifiers <i>SR Hashemi, I Zulkifli, M Hair-Bejo, TC Loh and H Davoodi</i>	103
22. Influence of L-leucine Supplementation in Varying Protein Levels on Sensory Meat Quality of Grower-Finisher Broiler <i>E Erwan, AR Alimon, AQ Sazili and H Yaakub</i>	106
23. Determination of Conjugated Linoleic Acid Content in Pasture and Palm Kernel Cake-Fed Kedah-Kelantan Cattle <i>I Hartini, AK Arifah, O Fauziah and A Abdul Razak</i>	110

24. Serological Surveillance of Q Fever in Goats, Cattle and Sheep in Malaysia <i>MM Arshad, R Mohamed, AN Hamid and AI Adzhar</i>	113
25. Ratio of Thyroxine and Triiodothyronine to Long Road Transportation of Bligon Bucks <i>P Astuti, Sarmin, A Kusumawati, CM Airin and L Sjahfirdi</i>	116
26. Motion-mode Echocardiography Reference Values in the Normal Mongrel Indonesian Dog <i>D Noviana, D Paramitha and R Wulansari</i>	123
27. Effect of <i>Channa striatus</i> Extract Against Monosodium Iodoacetate-Induced Osteoarthritis in the Rat <i>FJ Saffar S Ganabadi, S Fakurazi and H Yaacob</i>	124
28. Effect of Orally Administrated L-Arginine on Monosodium Iodoacetate-Induced Osteoarthritis in Rats <i>Nor Jawahir A., Ganabadi S., Arifah A. K. and Chen H.C</i>	128
29. Melatonin as Antioxidant Like in Experimental Diabetes Mellitus Type 1 <i>Boedi Setiawan, Hartanta Barus and Teresia Ayu Yuliamarini Banjarmasinor</i>	131
30. Surgical Approach for Treatment of Some Caseous Lymphadenitis in Goats <i>SK Tmumen</i>	135
31. Estrus Response of Goats Synchronized with Different Combinations of PGF ₂ α and Flugestone Acetate Sponges <i>MM Bukar, Y Rosnina, H Wahid, GK Dhaliwal, MA Omar, WZ Mohamed and GK Mohd Azam Khan</i>	136
32. The Influence of Eluate Fraction Sequence of Sephadex G-75 Separated 3.5 Month Pregnant Indonesian CBG4 Mares Serum on Estradiol-17 β Content <i>NMR Widjaja</i>	139
33. Sperm Concentration Determination: 2X- Cel Slide CASA vs Haemocytometer <i>N Yimer, Y Rosnina, A Wahid, AA Saharee, KC Yap and P Ganesamurthy</i>	144
34. Seminal Plasma Removal Increased Boer Semen Quality After Cryopreservation <i>SW Naing, H Wahid, GK Mohd Azam Khan, Y Rosnina, AB Zuki, J Audrey, N Fazmimi, K Wiwin, F Fakar, K Heri and IN Sapto</i>	148

24. Serological Surveillance of Q Fever in Goats, Cattle and Sheep in Malaysia 113
MM Arshad, R Mohamed, AN Hamid and AI Adzhar
25. Ratio of Thyroxine and Triiodothyronine to Long Road Transportation of Bligon Bucks 116
P Astuti, Sarmin, A Kusumawati, CM Airin and L Sjahfirdi
26. Motion-mode Echocardiography Reference Values in the Normal Mongrel Indonesian Dog 123
D Noviana, D Paramitha and R Wulansari
27. Effect of *Channa striatus* Extract Against Monosodium Iodoacetate-Induced Osteoarthritis in the Rat 124
FJ Saffar S Ganabadi, S Fakurazi and H Yaacob
28. Effect of Orally Administrated L-Arginine on Monosodium Iodoacetate-Induced Osteoarthritis in Rats 128
Nor Jawahir A., Ganabadi S., Arifah A. K. and Chen H.C
29. Melatonin as Antioxidant Like in Experimental Diabetes Mellitus Type 1 131
Boedi Setiawan, Hartanta Barus and Teresia Ayu Yuliamarini Banjarnahor
30. Surgical Approach for Treatment of Some Caseous Lymphadenitis in Goats 135
SK Tmumen
31. Estrus Response of Goats Synchronized with Different Combinations of PGF_{2α} and Flugestone Acetate Sponges 136
MM Bukar, Y Rosnina, H Wahid, GK Dhaliwal, MA Omar, WZ Mohamed and GK Mohd Azam Khan
32. The Influence of Eluate Fraction Sequence of Sephadex G-75 Separated 3.5 Month Pregnant Indonesian CBG4 Mares Serum on Estradiol-17β Content 139
NMR Widjaja
33. Sperm Concentration Determination: 2X- Cel Slide CASA vs Haemocytometer 144
N Yimer, Y Rosnina, A Wahid, AA Saharee, KC Yap and P Ganesamurthy
34. Seminal Plasma Removal Increased Boer Semen Quality After Cryopreservation 148
SW Naing, H Wahid, GK Mohd Azam Khan, Y Rosnina, AB Zuki, J Audrey, N Fazmimi, K Wiwin, F Fakar, K Heri and IN Sapto

Wild Type Rabies Virus Glycoprotein to Make Monoclonal Antibody for Early Detection with DAS-ELISA

Jola Rahmahani, Suwarno, Sri Susila Andayani and Kusnoto

Microbiology Departement Faculty of Veterinay Medicine

Airlangga University, Surabaya, Indonesia

E-mail : jola_rahmahani@yahoo.co.id

Abstract

The objective of this study was to search for wild type rabies virus glycoprotein G protein) for the production of monoclonal antibody for early detection with double antibody sandwich (DAS)-ELISA. This study was divided into several stages, including isolation and determination of type rabies virus with PCR, protein analysis with SDS-PAGE, immunogenicity and antigenicity tests, cloning with limiting dilution technique, purification of immunoglobulin, monoclonal antibody labeling, sensitivity and specificity test of monoclonal antibody used saliva and brain of mice with rabies virus infection. The result showed that the molecular weight of the G protein was 62–67 kDa. The result of cloning and recloning rabies virus showed that the virus was wild type (clone Jra 1–ra 8). Sensitivity values of monoclonal antibody used with DAS-ELISA from mice's brain was 95.23% and from mice's saliva was 97.62%. The specificity value of monoclonal antibody from mice's brain was 100% and from mice's saliva was 91.67%.

Keywords: rabies virus, G protein, monoclonal antibody and DAS-ELISA.

Introduction

Rabies is caused by a neurotropic virus belonging to genus *Lyssavirus*, family *Rhabdoviridae*, and is transmitted to all mammals. In Indonesia rabies is endemically distributed on the island of Sulawesi, Nusa Tenggara, Kalimantan and Sumatra. Vaccination and elimination program had been done, but the problem still uncovered. (Dibia, 2000; Sugiarto, 2001; Supriyadi *et al.* 2005)

Diagnosis to rabies cases, mainly in carnivora has many done by detection of antigen in brain samples (hippocampus, medulla oblongata, cortex cerebri or cerebellum) infected by rabies by the microscopic detection of negris bodies or via biologic test. This diagnosis by antigen detections is not practiced.

Antigen virus diagnosis can done with serologic tests, including indirect-FAT, virus neutralization, indirect ELISA, fluorescent antibodies virus neutralization (FAVN). (Kelly and Strik, 2000; Jackson *et al.*, 2000). In the field, these diagnostic methods are difficult to conduct because the wide geographic area of Indonesia (the islands of Sulawesi, Nusa Tenggara, Kalimantan and Sumatera).

This study attempted to produce a standard antigen diagnostic method of animal infected rabies using DAS-ELISA on saliva. DAS-ELISA used for antigen diagnosis is dependent on on sensitivity and specificity of monoclonal antibodies.

The objective of this study was to obtain wild type rabies virus glycoprotein for the production of monoclonal antibody to be used in early detection of the disease. The technique to be used is DAS-ELISA.

Methods

This study was divided on several stages, that is biological characterization of rabies virus in BHK – 21 cell lines, identification of virus by indirect ELISA and direct FAT, gene protein characterization by SDS – PAGE, and dot-blot technique. The Immunogenicity of protein coding was applied in mice. Molecular characterization by RT-PCR assays and analysis of PCR products was performed. Cloning and recloning monoclonal antibodies to G-protein wild type of rabies virus (Sambrook *et al.*, 1989; Suwarno *et al.*, 2002; Suwarno 2005).

Results and Discussion

Immunogenicity of G-protein is shown in Table 1. G-protein can induce antibodies titer reaching 10,240, while with whole molecule rabies virus the titre only reached 1,280. The high antibody titer with G-protein showed that it can be used to produce monoclonal antibodies.

Table 1: Immunogenicity of G-protein rabies virus to induce antibodies of mice by Indirect-ELISA

Kind of Antigen	Antibody Titre
G-protein	5120-10240
Whole molecule rabies virus	640-1280

Immunoglobulin subclass from monoclonal antibodies production indicated the success of cloning process. Immunoglobulin subclass from cloning and recloning is shown in Table 2.

Table 2: Immunoglobuline subclass to G-protein of rabies virus field isolates from cloning and recloning

Plate	Subclass Immunoglobulin		Clones name
	Cloning	Recloning	
1B2	Ig G1, Ig G2a	Ig G1	Jra-1
		Ig G2a	Jra-2
2D11	Ig G2b	Ig G2b	Jra-3
		Ig G2b	Jra-4
3H7	Ig M, Ig G3	Ig M	Jra-5
		Ig M	Jra-6
4G9	Ig G1, Ig G3	Ig G1	Jra-7
		Ig G3	Jra-8

References

- Dibia, IN. 2000. Surveillance and Destroyed Rabies in Flores NTT islands. Technical Meeting of Science Animal Health, Agriculture and Forestry, Bogor 5-6 October.
- Jackson, AC. 2000. Rabies. *Can. J. Neurol. Sci.* **27(4)**:278-282.
- Kelly, RM. and PL. Strick. 2000. Rabies as a transneuronal tracer of circuits in the central nerves system. *J. Neurosci. Methods.* **103(1)**: 63-71.
- Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular Cloning. 2nd Ed. Cold Spring Harbor Laboratory Press, New York.
- Sugiarto. 2001. Report Rabies Diagnostic Period 1996/2001 in BPPV area Regional VII Maros.
- Supriyadi, A., S. Hadi dan J.S. Kalianda. 2005. Freedom of Rabies in Kalimantan. Report rabies Kalimantan in Pontianak 25-27 July.
- Suwarno. 2005. Rabies Diagnostic with PCR technic. Short Course PCR technic for Rabies. BPPV Regional V Banjarbaru, 28-31 July.
- Suwarno, S. Sugijanto, dan F.A. Rantam. 2002. Produce monoclonal antibodies to isolate dengue virus Surabaya. *Media Kedokteran Hewan* **18 (2)**:64-68.