RESPON KEKEBALAN HUMORAL MENCIT BALB/C YANG DIVAKSINASI DENGAN VAKSIN LIMPA DAN VAKSIN KULTUR PENYAKIT JEMBRANA TERHADAP PROTEIN VIRUS JEMBRANA

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Abstrak

Sapi bali adalah salah satu aset nasional Indonesia yang harus dilestarikan karena mempunyai keuntungan ekonomi. Tetapi sapi bali mempunyai beberapa kelemahan penyakit khususnya penyakit Jembrana yang disebabkan oleh virus penyakit Jembrana (*JDV*). Pencegahan terhadap penyakit Jembrana telah dilakukan dengan vaksinasi. Vaksin yang terbukti dapat menurunkan tingkat kematian sapi bali terserang *JDV* adalah vaksin limpa. Jenis vaksin ini hanya mampu menginduksi kekebalan dengan perlindungan 70%. Proteksi ini dapat ditingkatkan jika jumlah virus yang digunakan dalam vaksin meningkat. Tekhnik kultur in vitro adalah salah satu metode meningkatkan jumlah virus penyakit Jembrana, dan selanjutnya dibuat vaksin kultur. Hasil penelitian menunjukkan bahwa sel limfosit sapi bali terinfeksi *JDV* adalah 9,5% pada limpa dan 57,43% pada sel kultur. Uji *westernimmunoblotting* sel limfosit sapi bali dari darah tepi dan limpa terinfeksi *JDV* menggunakan antibodi monoklonal (AbMo) anti *Ca*, terdeteksi protein dengan berat molekul 26 kDa, 42 kDa dan 51 kDa. Pada medium kultur *PBMC* dan endapan plasma sapi bali terinfeksi *JDV*, teridentifikasi protein dengan berat molekul 16 kDa an 26 kDa menggunakan *AbMo,* dan teridentifikasi protein dengan berat molekul 16 kDa; 21,5 kDa. 26 kDa; 29,7 kDa; 40 kDa dan 50 kDa menggunakan *AbPo*. Uji *Elisa* didapatkan nilai absorban antibodi mencit balb/c yang divaksinasi dengan vaksin kultur penyakit Jembrana lebih tinggi yaitu sebesar 0,3089 dibandingkan vaksin limpa yaitu sebesar 0,177 dengan $p<0,05$. Nilai absorban antibodi mencit balb/c terhadap antigen *Ca*, *SU* dan *tat*, memperlihatkan nilai absorban terhadap antigen *SU* berbeda sangat signifikan dibandingkan dengan antigen *Ca* dan antigen *tat* ($p<0,01$). Antigen *Ca* berbeda signifikan terhadap antigen *tat* ($p<0,05$).

Kata kunci : Virus penyakit Jembrana, sel limfosit sapi bali terinfeksi, uji *westernimmunoblotting*, uji *elisa*, vaksin limpa, vaksin kultur, antigen kapsid (*Ca*), antigen *surface unit* (*SU*), antigen transaktivator transkripsi (*tat*).

1. LATAR BELAKANG

Sapi bali merupakan spesies asli Indonesia yang harus dilestarikan dari kepunahan. Sapi bali mempunyai beberapa keuntungan antara lain sebagai penghasil

daging, sumber pendapatan, sebagai pekerja, penghasil pupuk untuk kesuburan tanah dan dapat bertahan pada musim kemarau. Tetapi sapi bali juga mempunyai beberapa kelemahan antara lain sensitif terhadap penyakit *malignant catharral fever* (*MCF*), baliziekte dan khususnya penyakit Jembrana.

Penyakit Jembrana yang disebabkan oleh virus penyakit Jembrana (*JDV*) tidak hanya menyerang sapi bali di propinsi Bali saja, tetapi kasusnya telah menyebar ke propinsi lainnya di Indonesia antara lain : propinsi Lampung (Lampung Tengah) dikenal dengan nama penyakit Rama Dewa (Prabowo dan Ishitani, 1984); propinsi Jawa Timur (kabupaten Banyuwangi) dikenal dengan nama penyakit Banyuwangi (Sudana *et al*., 1979); propinsi Sumatera Barat (kabupaten Sawahlunto Sijunjung); propinsi Kalimantan Selatan dan propinsi Bengkulu (kabupaten Bengkulu Selatan) (Wiryosuhanto, 1996).

Pencegahan terhadap penyakit Jembrana telah dilakukan dengan vaksinasi. Vaksin yang terbukti dapat menurunkan tingkat kematian sapi bali terserang *JDV* adalah vaksin limpa. Jenis vaksin ini menginduksi respon kekebalan dengan perlindungan 70%. Perlindungan vaksin yang tidak maximal ini, memungkinkan timbulnya infeksi baru pada sapi bali. Kurang memadainya tingkat proteksi yang diinduksi oleh vaksin limpa disebabkan oleh sedikitnya sel terinfeksi *JDV* dalam limpa yang digunakan untuk membuat vaksin. Proteksi ini dapat ditingkatkan jika jumlah virus yang digunakan dalam vaksin meningkat.Tekhnik kultur virus secara in vitro adalah salah satu metode meningkatkan jumalh virus terhadap penyakit Jembrana. Potensi dari vaksin kultur dengan mendeteksi respon kekebalan humoral terhadap berbagai jenis protein *JDV* diantaranya kapsid (*Ca*), *surface unit* (*SU*) dan transaktivator transkripsi (*tat*) belum pernah dilaporkan. Untuk mendeteksi antibodi terhadap protein *Ca*, digunakan protein rekombinan *Ca* (sebagai protein fusi dengan *histidine*) (Barboni *et al*., 2000), terhadap protein *tat* digunakan protein rekombinan *tat* yang diekspresikan oleh bakteri *Escherichia coli* (Setiyaningsih, 2006) dan terhadap protein *SU* digunakan protein rekombinan *SU.*

2. METODE PENELITIAN

2.1 Rancangan Penelitian

Rancangan penelitian yang dipergunakan dalam penelitian ini adalah rancangan acak lengkap pola berjenjang dengan rancangan petak-terbagi (*split-plot design*). Faktor utama adalah jenis vaksin dan faktor tambahan adalah jenis antigen. Rancangan eksperimental menggunakan *Randomized Post Test Only Control Group Design*.

2.2 Populasi dan Sampel

Populasi dalam penelitian ini adalah mencit balb/c, sedangkan sampel penelitian adalah mencit balb/c betina, berumur 2 bulan, dengan berat badan 20-30 gram (Malole, 1989).

2.3 Prosedur Penelitian

2.3.1 Sel kultur

Endapan limfosit normal yang diisolasi dari Peripheral Blood Limfosit Cells (PBLC) ditambahkan media *Dulbeco's Minimum Eagle Medium* (*DMEM*) yang menagandung 10% *Fetal Calf Serum* (*FCS*), 2 µg *ConA* dan *IL-2*. Kultur ini diinfeksikan *JDV* yang diperoleh dari sel limfosit terinfeksi *JDV* dari limpa pada demam hari kedua pasca inokulasi. Suspensi sel dimasukkan ke dalam biakan sel 96 sumuran dan diinkubasikan pada suhu 37^0C dengan kadar CO_2 lingkungan 5%. Identifikasi protein *JDV* dari sel limfosit terinfeksi pada kultur dilakukan dengan uji *westernimmunoblotting*.

2.3.2 Immunohistokimia dan *westernimmunoblotting*

Immunohistokimia mengikuti prosedur Dharma (1992) dan *westernimmunoblotting* mengikuti prosedur Astawa dalam Diagnosis Penyakit Jembrana (Hartaningsih, 2002).

2.3.3 Nilai Absorban Antibodi Mencit balb/c dengan Elisa

Pemeriksaan *Elisa* berdasarkan prosedur Hartaningsih (2002).

2.3.4 Analisa Data

Pengaruh vaksinasi vaksin limpa dan vaksin kultur penyakit Jembrana pada mencit balb/c dengan nilai absorban terhadap antigen *Ca, SU* dan *tat* dianalisis dengan analisis univariat menggunakan SPSS 13 (Trihendradi, 2005). Jika terdapat perbedaan bermakna antara ketiga antigen, dilanjutkan dengan uji *LSD* (Steel & Torrie, 1989) (Trihendradi, 2005). Untuk membandingkan nilai absorban antibodi mencit balb/c yang divaksinasi dengan vaksin limpa dan vaksin kultur penyakit Jembrana dilakukan dengan uji t (Sugiyono, 2007).

3. HASIL DAN PEMBAHASAN

3.1 Uji IHK dan Uji Westernimmunoblotting Sel-sel Limfosit Terinfeksi JDV dari Limfosit Limpa dan Darah tepi

Hasil uji imunoperoksidase (IHK) menggunakan antigen *Ca, SU* dan *tat* yang sudah dikarakterisasi, ditemukan sitoplasma sel limfosit sapi bali terinfeksi *JDV* berwarna coklat dan nukleus berwarna ungu. Sedangkan sitoplasma sel tidak terinfeksi *JDV* ditemukan berwarna transparan dengan nukleus berwarna ungu. Uji *westerimmunoblotting* menggunakan *AbMo anti-Ca,* sel limfosit sapi bali terinfeksi yang diperoleh dari limpa dan darah tepi, terdeteksi protein dengan berat molekul 26 kDa, 42 kDa dan 51 kDa. Uji *westernimmunoblotting* menggunakan *AbMo* dari endapan medium kultur *Peripheral Blood Mononuclear Cells* (*PBMC*) dan plasma sapi bali terinfeksi *JDV* teridentifikasi protein dengan berat molekul 16 kDa dan 26 kDa. Sedangkan dengan menggunakan *Antibodi poliklonal* (*AbPo*) pada kultur *PBMC* dan endapan plasma sapi bali terinfeksai *JDV*, teridentifikasi protein denagan berat molekul 16 kDa, 21,5 kDa; 26 kDa; 29,7 kDa; 40 kDa dan 50 kDa.

3.2 Nilai Absorban Antibodi Mencit Balb/c yang Divaksinasi dengan Vaksin Limpa dan Vaksin Kultur Penyakit Jembrana

Nilai absorban antibodi mencit balb/c yang diavaksinasi dengan vaksin limpa dan vaksin kultur penyakit Jembrana dilakukan dengan uji Elisa, menggunakan protein Ca, protein SU dan protein tat. Reaksi biokimia dari uji Elisa ini adalah reaksi warna,

dimana reaksinya memperlihatkan warna biru jika terjadi reaksi enzim dengan substrat. Rata-rata nilai absorban antibodi mencit balb/c yang divaksinasi dengan vaksin limpa penyakit Jembrana adalah sebesar 0,15 terhadap antigen *Ca*; 0,36 terhadap antigen *SU* dan 0,02 terhadap antigen *tat*. Sedangkan rata-rata nilai absorban antibodi menggunakan vaksin kultur penyakit Jembrana terhadap antigen *Ca, SU* dan *tat* berturut-turut sebesar 0,18; 0,75; dan sebesar -0,00.

Hasil statistik univariat antibodi mencit balb/c menunjukkan bahwa jenis vaksin berpengaruh nyata terhadap nilai absorban antibodi mencit balb/c ($p<0,05$). Jenis antigen berpengaruh sangat nyata terhadap nilai absorban antibodi mencit balb/c yang divaksinasi dengan vaksin limpa dan vaksin kultur penyakit Jembrana ($p<0,01$). Terdapat interaksi yang nyata antara jenis vaksin dan jenis antigen ($p<0,05$). Uji t didapatkan hasil bahwa nilai absorban antibodi mencit balb/c yang divaksinasi dengan vaksin kultur lebih tinggi dibandingkan dengan vaksin limpa penyakit Jembrana (0,3089 : 0,177) dengan $p<0,05$. Varian yang didapatkan adalah sebesar 5,156, yang berbanding lurus dengan tingkat sel limfosit terinfeksi pada sapi bali dari limpa dibandingkan dengan kultur sel (9,5% : 57,43%). Nilai absorban terhadap protein *Ca, SU* dan protein tat memperlihatkan pada vaksin kultur, nilai absorban antibodi terhadap antigen *SU* lebih tinggi dibandingkan dengan vaksin limpa penyakit Jembrana ($p<0,01$). Sedangkan terhadap antigen *Ca* dan antigen tat tidak menunjukkan perbedaan yang bermakna baik pada vaksin limpa maupun vaksin kultur penyakit Jembrana ($p>0,05$). Uji *LSD*, didapatkan bahwa nilai absorban antibodi dengan antigen *SU* berbeda sangat signifikan dengan antigen *Ca* dan antigen *tat* ($p<0,01$). Sedangkan antigen *Ca* dan antigen *tat* berbeda signifikan ($p<0,05$).

Hasil uji *Elisa* menunjukkan bahwa vaksin kultur memberikan respon kekebalan humoral yang secara bermakna lebih tinggi daripada vaksin limpa penyakit Jembrana dengan $p<0,05$. Hasil penelitian ini sejalan dengan penelitian yang dilakukan oleh Sullivan *et al* ., (1998); dan Kortrikis *et al.,* (1996) bahwa antibodi terhadap *HIV-1* dapat meningkatkan replikasi virus dalam model sel kultur.

Hasil penelitian ini menunjukkan bahwa respon kekebalan humoral oleh antigen *SU* pada vaksin kultur lebih tinggi dibandingkan dengan vaksin limpa penyakit

Jembrana. Antibodi terhadap antigen *SU* lebih tinggi dibandingkan dengan *Ca* dan antigen *tat.* Penelitian pada sapi, ditemukan antigen *Ca* yang imunodominan (Kertayadnya *et al.*, 1993., Zheng *et al.*, 2001). Sedangkan pada penelitian ini dengan menggunakan mencit balb/c ditemukan antigen *SU* imunodominan. Hasil penelitian yang sama (*SU* imunodominan) juga ditemukan oleh Hermann *et al* (2005) pada *Ovine Progressive Pneumonia Virus* (*OPPV*) yang menginfeksi domba, Ball *et al* (1992), Issel (1997), Issel dan Cook (1993), Lonning *et al* (1999) yang melakukan penelitian pada *EIAV* (*Equine Infectious Anemia Virus*); Mankowski *et al* (1997) yang meneliti pada *SIV* (*Simmian Immunodeficiency Virus*) dan Richardson *et al* (2002) yang meneliti *FIV* (*Feline Immunodeficiency Virus*) pada kucing. Disamping perbedaan spesies (sapi vs mencit balb/c), pada penelitian ini juga diberikan imunisasi yang berulang. Dengan demikian, kemungkinan faktor spesies dan juga pemberian vaksin yang berulang berpengaruh terhadap respon kekebalan humoral.

4. SIMPULAN, KEBAHARUAN DAN SARAN

4.1 Simpulan

1. Protein khas *JDV* yang terdeteksi pada sel limfosit asal limpa terinfeksi sama dengan sel limfosit darah tepi dengan berat molekul 26 kDa, 42 kDa dan 51 kDa menggunakan *AbMo* terhadap protein *Ca*

2. Protein khas *JDV* terdeteksi pada endapan medium kultur *PBMC* sama dengan pada endapan plasma sapi terinfeksi *JDV* dengan berat molekul 26 kDa dan 16 kDa menggunakan *AbMo*. Sedangkan dengan menggunakan AbPo, terdeteksi protein dengan berat molekul 16 kDa; 21,5 kDa; 26 kDa; 29,7 kDa; 40 kDa dan 50 kDa baik dari kultur *PBMC* dan endapan plasma sapi bali terinfeksi *JDV*
3. Rata-rata nilai absorban antibodi mencit balb/c yang divaksinasi dengan vaksin kultur lebih tinggi dibandingkan dengan vaksin limpa penyakit Jembrana, dan perbedaan ini bermakna secara statistik ($p<0,05$).
4. Nilai absorban antibodi terhadap antigen *SU* lebih tinggi dibandingkan dengan nilai absorban antibodi terhadap antigen *Ca*, baik pada mencit balb/c yang

divaksinasi dengan vaksin limpa maupun vaksin kultur penyakit Jembrana ($p<0,01$). Dengan demikian antigen *SU* imunodominan

5. Nilai absorban antibodi mencit balb/c yang divaksinasi dengan vaksin kultur terhadap antigen *SU* lebih tinggi dibandingkan dengan nilai absorban antibodi mencit balb/c yang divaksinasi dengan vaksin limpa penyakit Jembrana ($p<0,01$).
6. Nilai absorban antibodi mencit balb/c terhadap antigen *SU* lebih tinggi dibandingkan dengan nilai absorban antibodi terhadap antigen *Ca* dan antigen *tat* ($p<0,01$), nilai absorban antibodi terhadap antigen *Ca* lebih tinggi dibandingkan dengan nilai absorban antibodi terhadap antigen *tat* ($p<0,05$), baik pada mencit yang divaksinasi dengan vaksin limpa dan vaksin kultur penyakit Jembrana.
7. Nilai absorban antibodi terhadap antigen *SU* lebih tinggi dibandingkan dengan *Ca* karena pemberian vaksinasi berulang pada mencit yang divaksinasi dengan vaksin limpa dan vaksin kultur penyakit Jembrana

4.2 Kebaharuan

1. Mencit balb/c mampu merangsang respon kekebalan humoral terhadap vaksin limpa dan vaksin kultur penyakit Jembrana
2. Nilai absorban antibodi mencit balb/c yang divaksinasi dengan vaksin kultur lebih tinggi dibandingkan dengan vaksin limpa penyakit Jembrana.
3. Vaksinasi berulang pada mencit balb/c akan meningkatkan antibodi antibodi *SU JDV*

4.3 Saran

1. Mencit balb/c dapat digunakan sebagai hewan model untuk uji coba efikasi vaksin *JDV*.
2. Perlu dilakukan vaksinasi secara berulang untuk merangsang antibodi protein *SU*
3. Perlu dipertimbangkan pembuatan vaksin kultur dalam limfosit kultur untuk meningkatkan jumlah *JDV*
4. Perlu diupayakan cara untuk menumbuhkan limfosit kultur sapi bali tanpa menggunakan *IL-2* (untuk menekan biaya produksi)

HUMORAL IMMUNE RESPONSE OF BALB/C MICE VACCINATED WITH JEMBRANA DISEASE SPLEEN AND CULTURE VACCINE AGAINST JEMBRANA VIRAL PROTEINS

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Abstract

Bali cattle are one of Indonesia's national assets that have to be properly concerved because they have economic advantages. However, Bali cattle have some weaknesses such as they are vulnerable to some diseases, particularly Jembrana Disease caused by Jembrana Disease Virus. Prevention of Jembrana Disease has been done by vaccination. The vaccine that has been proven to be capable to reduce the mortality rate of Bali cattle suffering from Jembrana Disease Virus (JDV) is spleen vaccine. This type of vaccine is only able to induce immune response with 70% level of protection. This protection can be improved if the number of viruses used in the vaccine increases. In vitro virus culture technique is one of the methods to increase the number of viruses, and then culture vaccine is made. This research shows that JDV infected Bali cattle lymphocyte cells were 9, 5% in spleen and 57, 43% in culture. The westernimmunoblotting using monoclonal (MoAb) anti-Ca antibody infected Bali cattle lymphocyte cells obtained from the spleen and peripheral blood protein with molecule weight of 26 kDa, 42 kDa and 51 kDa was detected. In Peripheral Blood Momonuclear Cell (PBMC) pellet medium culture and JDV plasma protein with molecular weight of 16 kDa and 26 kDa using AbMo, and using polyclonal antibody proteins with molecular weight of 16 kDa; 21,5 kDa; 26 kDa; 29,7 kDa; 40 kDa and 50 kDa was identified. Elisa tes found out that absorbent value of balb/c mice antibody with JDV culture vaccine was higer i.e 0.3089 compared with that of JDV spleen vaccine =0.177 at $p<0.05$. The absorbent values of balb/c mice antibody against Ca, SU and tat proteins show that the antibody absorbent value against SU antigen was really significantly different from that against Ca and tat antigen ($p<0.01$). Ca antigen was significantly different from tat antigens ($p>0.05$).

Key words: Jembrana Disease Virus, infected Bali cattle lymphocytes cells, westernimmunoblotting test, elisa test, spleen vaccine, culture vaccne, Capsid (Ca) antigen, surface unit (SU) antigen, transactivation transcription (tat) antigen.

1.BACKGROUND

Bali cattle are Indonesia's original species which have to be properly concerved. Bali cattle are considered as original species with several advantages, such as they

produce meat, generate income, function as worker, produce fertilizer for soil fertility and are able to survive during dry season. However, Bali cattle have some weaknesses such as they are vulnerable against Malignant Catarharral Fever (MCF), baliziekte and particularly Jembrana Disease.

Jembrana disease does not attack bali cattle in Bali province only, but it has also spread to other provinces in Indonesia, such as Lampung province (Central Lampung), where it is known as Rama Dewa Disease (Prabowo & Ishitani, 1984); East Java (Banyuwangi regency), locally known as Banyuwangi Disease (Sudana et al., 1979); West Sumatera province (Sawahlunto Sijunjung regency); South Kalimantan province and Bengkulu province (Wiryosuhanto, 1996).

Prevention of Jembrana Disease has been done by vaccination. The vaccine that has been proven to be capable to reduce the mortality rate of Bali cattle suffering from Jembrana Disease Virus (JDV) is spleen vaccine. This type of vaccine induces immune response with 70% level of protection. The insufficient protection of this vaccine makes it possible for Bali cattle to be reinfected. The inadequate level of protection induced by spleen vaccine is due to the number of JDV infected cells in the spleen used to produce the vaccine is very limited. This protection can be improved if the number of viruses used in the vaccine increases. In vitro virus culture technique is one of the methods to increase the number of viruses to protect the cattle against Jembrana Disease. The potential of JDV culture vaccine by detecting the humoral response to various types of JDV protein such as capsid (Ca), surface unit (SU) and transcription transactivator (tat) has never been reported. Recombinant Ca protein is used to detect antibody against Ca protein (as fusion of protein with histidine) (Barboni et al 2000); recombinant tat protein expressed by *Escherichia coli* is used to detect antibody against tat protein (Setyaningsih, 2006), and recombinant protein is used to detect antibody against SU.

2. RESEARCH METHODS

2.1 Research Design

The research designs used in this study were stratified complete random design and split-plot design. The main factors in this study were the types of vaccine and the additional factors were the types of antigen. The experimental study referred to randomized post test only control group design.

2.2 Population and Sample

The Population in this research was balb/c mice, while the sample was female balb/c mice, two months old, with body weight of 20-30 gram (Malole, 1989).

2.3 RESEARCH PROCEDURE

2.3.1. Culture Cell

The normal lymphocytes of Bali cattle were obtained from centrifuged Peripheral Blood Lymfocyte Cells (PBLC). By this method lymphocyte pellets were obtained. Dulbeco's Minimum Eagle Media (DMEM) containing 10% Fetal Calf Serum (FCS), 2 µg Concanavalin A (Con-A) and interleukin 2 (IL-2) was added to the normal lymphocyte pellets. This culture was infected with JDV obtained from JDV infected lymphocyte spleen cells from the time when cattle suffered from fever two days after inoculation. The cell suspension was put in cell multiplication micro-plates of 96 wells and incubated at 37^0C with 5% CO_2 environment content. Westernimmunoblotting test was done to identify the JDV protein of infected lymphocytes in the culture.

2.3.2. Immunohistochemistry and Westernimmunoblotting

Immunohistochemistry (immunoperoxidase test) was done according to the Dharma's procedure (1992) and westernimmunoblotting was done following Astawa's procedure in Jembrana Disease Diagnosis (Hartaningsih, 2002).

2.3.3 Absorbent Value of Balb/c Mice Antibody by ELISA

Elisa test was done following Hartaningsih (2002).

2.3.4 Data Analysis

The effect of JDV spleen vaccine and culture vaccine in balb/c mice with the absorbent level against Ca, SU and tat antigen was analyzed by univariate analysis using SPSS 13 (Trihendradi, 2005). If there was a significant difference, it was followed by test of Least Significant Difference (LSD) (Steel & Torrie, 1989) (Trihendradi, 2005). To compare JDV spleen vaccine and culture vaccine, t test was used (Sugiyono, 2007).

3. RESULTS AND DISCUSSION

3.1 Immunohistochemistry and westernimmunoblotting test on lymphocyte cells of spleen lymphocyte and peripheral blood infected by JDV

The result of study by immunopexidase (immunohistochemistry) test using antigen Ca, SU and tat that had been characterized showed that JDV infected Bali cattle lymfocyte cell sitoplasm was brown in color , while the unifected one was transparent with bluish purple nucleus. The westernimmunoblotting using monoclonal antibody (MoAb) anti-Ca, infected Bali cattle lymphocyte cells obtained from the spleen and peripheral blood was detected to contain protein with molecule weight of 26 kDa, 42 kDa and 51 kDa. The westernimmunoblotting test using AbMo, Peripheral Blood Momonuclear Cell (PBMC) pellet medium culture and JDV, infected plasma was identified to contain protein with molecular weight of 16 kDa and 26 kDa. On the other hand, by using polyclonal antibody from PBMC medium culture, JDV infected bali cattle plasma pellet was identified to contain proteins with molecular weight of 16 kDa; 21,5 kDa; 26 kDa; 29,7 kDa; 40 kDa and 50 kDa.

3.2 Absorbent Values of the Antibody of Balb/c Mice Vaccinated by JDV Spleen Vaccine and Culture Vaccine

To find out the absorbent values of antibody of balb/c mice vaccinated by JDV spleen vaccine and culture vaccine, Enzym Linked Immunosorbent Assay (ELISA) test was used. In this case, Ca, SU and tat proteins were used. The biochemical reaction of this Elisa test was in the form of color reaction, in which the reaction showed blue color

when enzyme reacted to the substrate. The averages of the absorbent value of balb/c mice vaccinated by JDV spleen vaccine antibody were 0.15 against Ca antigen; 0.36 against SU antigen; and 0.02 against tat antigen. On the other hand,the averages of the absorbent value of antibody using JDV culture vaccine against Ca, SU and tat antigens were 0.18; 0.75 and -0.00 respectively.

Univariat analysis of antibody balb/c mice showed that the types of vaccine significantly influenced the absorbent value of antibody balb/c mice (p<0, 05).The types of antigen extremely significantly influenced the absorbent value of antibody of balb/c mice vaccinated with spleen vaccine and culture vaccine JDV (p<0, 01). Significant interaction between the types of vaccine and types of antigen (p<0, 05) was seen. T test showed that antibody absorbent value balb/c mice vaccinated JDV culture vaccine was higer than spleen vaccine (0, 3089: 0,177) at p<0, 05. The variance was 5,156, which was proportional to the degree of infected lymphocytes in spleen compared with culture cells of Bali cattle (9,5% : 57,43%). The absorbent value against Ca, SU and tat proteins showed that in culture vaccine the antibody absorbent value against SU antigen was higher than that against JDV spleen vaccine (p<0,01). On the other hand, the absorbent values against Ca and tat antigens did not show a significant difference both in JDV spleen vaccine and culture vaccine (p>0.05). The LSD test that followed shows that the antibody absorbent value against SU antigen was very significantly different from that against Ca and tat antigen (p<0, 01). On the other hand, Ca antigen and tat antigen were significantly different (p<0, 05).

Elisa test showed that JDV culture vaccine gave a significantly higher response than JDV spleen vaccine at p<0.05. The result of this study showed a similar result to that of Sullivan et al (1998) and that of Kortrikis et al (1996) that antibody against HIV-1 can increase virus replication in culture cell model.

The result of this study showed that the antibody against SU antigen in JDV culture vaccine was higher than that against spleen vaccine. Antibody against SU antigen was higher than Ca and tat antigen. In the study on cattle, immunodominant Ca antigen was found (Kertayadnya et al., 1993. Zheng et al, 2001). On the other hand, in this study using balb/c mouse, immunodominant SU antigen was found. This result is

the same as that of the study conducted by Hermann et al (2005) in Ovine Progressive Pneumonia Virus (OPPV) who infected goat, Ball et al (1992), Issel (1997), Issel and Cook (1993), Lonning et al (1999) who conducted a research on EIAV (Aquine Infectious Anemia Virus), Mankowski et al (1997) who conducted a research on SIV (Simmian Immunodeficiency Virus) and Richardson et al (2002) who conducted a research on FIV (Feline Immunodeficiency Virus) in cats. In addition to the difference in species (cattle vs balb/c mice), repeated vaccination was also given in this study. Thus, it is possible that species factor and repeated vaccination contributed to the humoral immune response.

4. CONCLUSION, NOVELTY AND SUGGESTIONS

4.1 Conclusions

1. Specific JDV protein detected in lymphocyte cells where the infected spleen came from is the same as peripheral blood lymphocyte cells with the molecular weight of 26 kDa, 42 kDa and 51 kDa using AbMo against Ca protein.
2. JDV specific protein detected in pellet medium culture PBMC is the same as that in pellet plasma of JDV infected cattle with the molecular weight of 26 kDa and 16 kDa by using AbMo. On the other hand, by using AbPo, protein with the molecular weight of 16 kDa, 21.5 kDa, 29.7 kDa, 40 kDa and 50 kDa both from culture PBMC and pellet plasma of JDV infected bali cattle was detected
3. The average absorbent value of balb/c mice vaccinated with culture vaccine is higher than that with JDV spleen vaccine, and the difference is statistically significant ($p<0.05$).
4. The absorbent value of the antibody against SU antigen is higher than that against Ca antigen, both in balb/c mice vaccinated with spleen vaccine and JDV culture vaccine ($p<0.01$). Thus SU antigen is immunodominant.
5. The absorbent value of balb/c mice antibody vaccinated with culture vaccine against SU antigen is higher than that vaccinated with spleen vaccine ($p>0.01$).

6. The absorbent value of antibody in balb/c mice against SU antigen is higher than that against Ca and tat antigens ($p<0.01$), the absorbent value of antibody against Ca antigen is higher compared with that in tat antigen ($p<0.05$), both in mice vaccinated with spleen vaccine and that vaccinated with JDV culture vaccine.
7. The higher absorbent value against SU antigen compared with that against Ca antigen is caused by repeated vaccination of vaccine in mice vaccinated with spleen vaccine and JDV culture vaccine.

4.2 Novelty

1. Balb/c mice is capable to stimulate the humoral immune response against Jembrana Disease spleen and culture vaccine
2. The absorbent value of antibody of balb/c mice vaccinated with culture vaccine is higher than that with JDV spleen vaccine
3. Repeated vaccination in balb/c mice will increase the SU antibody of JDV

4.3 Suggestions

1. The balb/c mice can be used as animal model for efficacy JDV vaccine trial
2. Repeated vaccination is needed to stimulate the production of SU antibody protein
3. The production of JDV culture vaccine in lymphocyte culture to increase the quantity of JDV should be taken into consideration
4. A method to grow the Bali cattle lymphocyte culture without using IL-2 (to minimize cost production) should be searched for.

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