RINGKASAN

Isolasi dan Karakterisasi Protein Imunogenik *Egp 51* Virus EBL Isolat Lokal Sebagai Kandidat Vaksin Sub Unit

Penelitian Eksperimental Laboratoris

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Penyakit *Enzootic Bovine Leucosis* (EBL) disebut pula *Lymphocytomatosis, Lymphosarcoma* atau Leukemia Sapi. Penyakit EBL disebabkan oleh *Retrovirus Exogenoses*. Secara structural dan fungsional menyerupai *Human T-Limphotropic Virus-1 (HTLV-1)* dan *Human T-Limphotropic Virus-2 (HTLV-2)*. Virus EBL menyerang sistem *Reticulo Endhothelial System (RES)*; sasaran utama virus EBL adalah limfosit B yang menyebabkan adanya proliferasi dan differensiasi abnormal sel-sel limfosit secara cepat yang mengakibatkan timbulnya benjolan-benjolan pada seluruh *limphoglandulae* dan alat-alat *viscera* seperti jantung yang mengakibatkan pembesaran jantung, pembesaran lambung, pada hepar menimbulkan hepatomegali, dan bila bagian tersebut diiris akan mengeluarkan nanah (pus) yang berwarna putih kekuningan, dan mengeras; yang merupakan masa tumor onkogenik dan menyebabkan aroma (bau) yang tidak sedap pada daging sapi. Pada sapi betina yang bunting dapat menyebabkan kematian janin dan pada sapi jantan menyebabkan timbulnya infertilitas (OIE 2003, Nakajima, *et.al*, 2000). Virus EBL bersifat persisten, dimana sekali saja virus EBL menginfeksi hewan ternak sapi, maka seumur hidup virus EBL akan tumbuh dan berkembang di tubuh sapi tersebut, dan akhirnya sapi mati karena penyakit EBL.

Penyakit EBL berjalan sub klinis dan menimbulkan kematian secara mendadak, angka kematian tinggi mencapai 90% - 100% dan menyebabkan kerugian ekonomi yang tinggi pula (Ressang, 1986, Ditkeswan, 1996).

Penyakit EBL adalah termasuk golongan penyakit Daftar A dan bersifat endemik, yaitu penyakit menular yang mempunyai potensi penyebaran yang sangat serius dan cepat, melewati batas negara yang menyebabkan konsekuensi yang serius terhadap sosio ekonomi atau kesehatan masyarakat dan kepentingan umum pada perdagangan hewan secara internasional (Dinas Peternakan Jatim, 2004; OIE, 2003).

Virus EBL merupakan *retro virus* onkogenik eksogenus yang *berenvelop*, dibawah elektron mikroskop berupa partikel *tipe-C*, mempunyai *buoayant density* 1,18g/ml. Virus EBL terdiri dari *RNA* 60S-70S yang berantai tunggal (*Single stranded)* serta memiliki enzim *reverse transcriptase*

Virus EBL menginfeksi hewan ternak melalui gigitan vektor lalat $Tabanus_{sp}$ dan *Hippobosca meliensis* masuk ke dalam kulit, sub epidermis dan aliran darah tepi

menuju limfosit B dan akhirnya menuju *limphoglandulae* yang berada di seluruh tubuh.

Penanganan selama ini hanya dengan diagnosis melalui uji *screening* menggunakan antigen KIT *Agar Gel Immunodifusi* produk Canada, Australia dan Perancis, yang harganya cukup mahal. Penelitian pembuatan antigen KIT test penyakit EBL isolat Indonesia telah berhasil dibuat, yang hasilnya sama dengan KIT test produk Canada (Hasdianah, 2000). Selama ini untuk pengujian terhadap penyakit EBL telah dikerjakan oleh BPPH di seluruh wilayah Indonesia, dan cara penanganan yang lain adalah dengan mengadakan karantina ketat. Diagnosis yang sering digunakan sesuai dengan pertemuan ahli EBL di Copenhagen, Denmark 1997, adalah menggunakan *Agar Gel Immunodifusi* melalui uji *Ouchterlony*; dihasilkan garis presipitasi yang berwarna putih sebagai hasil ikatan antigen, antibodi spesifik di dalam agar *outchterlony*. Hasil positip garis putih diduga adanya infeksi virus EBL (Nossal, 1998; Ishino, *et.al*, 2000 ; Nakajima, *et.al*, 2000; OIE, 2003).

Antigen KIT Produk *import*, disamping harganya cukup mahal juga memerlukan waktu cukup lama untuk sampai di tempat tujuan, apalagi bila tempat tujuan di daerah terpencil, terkadang sapi sudah mati, antigen KIT belum juga tiba.

Kejadian endemik dapat pula terjadi mengingat kurangnya kesadaran dari para peternak untuk melaporkan adanya kematian sapi secara mewabah pada instansi-instansi yang terkait; adanya transmisi dapat terjadi fausta negatif, sangat berbahaya disebabkan tidak diketahui bila sapi tersebut telah positip EBL, akhirnya dapat menyebabkan penularan tinggi dan peletupan wabah serta penyakit ini bersifat endemik disamping itu keterbatasan dana untuk mendeteksi keberadaan penyakit EBL secara menyeluruh di seluruh daerah yang diduga dapat terjangkitnya penyakit EBL. Bertitik tolak dari uraian diatas, maka mendorong peneliti untuk dapat meneliti pembuatan kandidat vaksin sub unit protein imunogenik *Egp 51* virus EBL isolat lokal (lapangan).

Pembuatan protein imunogenik *Egp 51* virus EBL isolat lokal sebagai kandidat vaksin dimulai dengan persiapan media penumbuh virus EBL, yaitu *Cell Line Ovine Lung (OL)* yang dibuat dari paru-paru domba muda berumur 4 minggu yang didapat dari Rumah Potong Hewan (RPH) Pegirian Surabaya. Pada biakan *Cell OL* diinokulasikan virus EBL pasasi lanjut (pasase 16) yang berasal dari virus EBL isolat lapangan dari daerah Lembang, Jawa Barat, yang telah dikembangkan sebelumnya, pada pasase 15 dari BPMSOH Gunung Sindur, Bogor.

Virus EBL diadaptasikan sampai pasase 29, sambil dititrasi untuk mendapatkan virus yang sudah adaptasi dalam biakan *Cell OL* serta mempunyai titer yang tinggi yaitu $10^{7,8}$ $TCID_{50}$ sebagai kandidat vaksin sub unit *protein Egp 51*. Uji *Postulat Koch* untuk menentukan apakah virus EBL mempunyai sifat yang berbeda dengan virus alami setelah dilakukan pasase sampai 29 kali pada *Cell OL*. Untuk pembuktian adanya virus EBL dilakukan Uji *Postulat Koch* klasik yang terdiri pembuatan *cell line* OL, Uji Patologi Anatomi, dan identifikasi *cytopathogenic effect* (CPE). Pembuatan antigen KIT diagnosis penyakit EBL telah pula membuktikan keberadaan virus EBL, terbukti dari hasil uji serum lapangan terdapat garis presipitasi pada agar *ouchterlony* pada serum sapi yang diduga positif terinfeksi penyakit EBL yang dibandingkan dengan menggunakan antigen KIT diagnosis penyakit EBL

produksi Kanada sebagai kontrol; hasilnya kurang lebih sama (Thesis, Hasdianah, 2000).

Uji *Postulat Koch* Molekuler yang terdiri dari Uji AGID dan Uji *Western blott*. Hasil pasasi virus EBL dengan titer tinggi $10^{7.8}$ $TCID_{50}$ kemudian diadakan sonikasi dengan alat sonikator untuk mendapatkan bagian *envelope (E)* virus EBL yang mengandung protein *Egp 51*. Karakterisasi dengan SDS-PAGE, didapatkan beberapa macam protein EBL yang terdiri dari protein 15 kDa, 24 kDa, 30 kDa dan *envelope glycoprotein gp 51* kDa. Untuk mendapatkan *protein Egp 51*, langkah berikutnya diadakan elektro elusi, kemudian dilanjutkan dengan Uji Hemaglutinasi untuk membuktikan apakah protein E gp 51 mempunyai daya aglutinasi terhadap darah merah sapi yang merupakan protein hemaglutinin; yang dilanjutkan dengan Uji hambatan hemaglutinasi. Untuk membuktikan protein E gp 51 bersifat imunogenik dan protektif dilakukan Uji Respon Imun melalui Uji Serum Netralisasi, Uji *Western blott* dilanjutkan dengan Uji Imunositokimia; dan untuk mengetahui daya protektifitas dari protein *Egp 51* dilakukan Uji *In Vitro* dan Uji *In Vivo* menggunakan hewan coba kelinci dari Batu Malang. Uji *Postulat Koch* molekuler dengan Uji *immunoblotting* melalui *Western blott* dihasilkan satu *band* murni *protein Egp 51*. Pembuatan antibodi poliklonal, dengan menyuntikkan virus EBL pada hewan percobaan kelinci yang ditambah dengan *Freund Adjuvant Incomplete (FAI)* dan *Freund Adjuvant Complete (FAC)*, dihasilkan antibodi poliklonal yang digunakan untuk pengujian *Immunorespon* secara *invitro* yang terdiri dari uji *Haemaglutinasi*, uji hambatan *hemaglutinasi*, uji *imunositokimia* serta uji *Agar Gel Imunodiffusi*. Langkah berikutnya untuk mengetahui daya immunogenik dan protektif, diadakan uji *in vivo* dengan menggunakan hewan kelinci sebanyak 50 ekor (jenis kelamin jantan) dan berat badan rata – rata 4 kg. Pengujian *invivo* terdiri dari kelompok I sebagai kelompok kontrol, yang tidak diberi *protein Egp 51* tapi *dichallenge* dengan virus ganas titer $10^{7.8}$ $TCID_{50}$, kelompok IIa, IIb adalah kelompok yang diberi kandidat vaksin *protein Egp 51* dan dibooster masing-masing; satu kali, untuk kelompok IIa (P2) dan di*booster* 2 kali untuk kelompok IIb (P3), masing-masing pada minggu kedua, sedangkan kelompok IIc adalah kelompok yang *dichallenge* pada minggu ketiga dengan virus ganas titer $10^{7.8}$ $TCID_{50}$. Dari hasil tersebut terlihat bahwa pada kelompok yang tidak diberi kandidat vaksin sub unit *protein Egp 51* hewan coba mati semua, dan setelah diseksio terlihat benjolan-benjolan di seluruh *limphoglandulae*, alat *viscera* yaitu pembesaran lambung, jantung dan terdapat hepatomegali. Bila bagian benjolan tersebut diiris akan keluar nanah (pus) yang merupakan massa tumor EBL onkogenik; dan pada kelompok IIa, IIb, dan IIc, hewan coba semuanya hidup.

Dari hasil penelitian ini dapat ditarik kesimpulan bahwa virus EBL isolat lokal mengandung *sub unit protein Egp 51* yang merupakan protein hemaglutinin dan protein hemaglutinin *Egp 51* virus EBL isolat lokal bersifat imunogenik dan protektif.

Summary

Isolation And Characterization Of Immunogenic Protein *Egp 51* Of Local Isolate EBL Virus As Sub Unit Vaccine Candidate

Experimental Laboratories Research

Hasdianah Hasan Rohan

Enzootic Bovine Leucosis (EBL) disease is *Lymphocytomatosis Lymphosarcoma* or Cow Leukemia. EBL disease is because of *Retrovirus Exogenoses*. Structurally and functionally, it looking likes *Human T-Lymphotropic Virus-1 (HTLV-1)* and *Human T-Limphotropic Virus-2 (HTLV-2)*. EBL virus attacks *Reticulo Endhotelial System (RES)*; the main target of EBL virus is lymphocyte B which quickly result in proliferation and abnormal differentiate of lymphocyte cells which result in incidence of bumps on all *limphoglandulae* and viscera tools such as heart which result in heart swelling, stomach swelling, on hepar result in hepatomegall, and if the pieces is sliced will release white, yellowish, and ossifying pus; it constitutes mass of oncogenic tumor and result in indelicate aroma on beef. On female pregnant ox, it can cause the death of foetus and on male ox can cause infertility (OIE 2003, Nakajima, et al, 2000). EBL virus has persistent characteristic, where once EBL virus infect ox, they will grow and develop in body of the ox and finally the ox will death because of EBL disease.

EBL disease is sub clinic disease and can cause death soon. The death rate is high up to 90-100% and cause high economic loss too (Reesang, 1986; Ditkeswan, 1996).

EBL disease can be classified as disease of *List A* and has characteristic of endemic, that is, contagion which has spreading potential serious and fast getting through country boundaries and cause serious consequences to socioeconomic or public health and general importance on international commerce of livestock animal (Dinas Peternakan Jatim, 2004; OIE, 2003).

EBL virus is *enveloped retro virus*, on electronic microscope it is type-C particle and has *buoayant density* 1.18 gr/ml. EBL virus consists of RNA 60S-70S with single stranded and has *reverse transcriptase enzyme*.

EBL virus infects livestock animal through bite of vector fly *Tabanus* and *Hypobosca Milensis* enters into skin, sub epidermis and edge blood stream to lymphocyte B and finally to *lymphoglandulae* in all bodies.

During the time, the treatment is only with diagnosis through *screening test* by using antigen KIT *Agar Gel Immunodifusi* product of Canada, Australia, and French with high price. Research to product antigen KIT test of isolate EBL disease in Indonesia has been success with the result of product is same with KIT Test Product of Canada (Thesis, Hasdianah, 2000). During the time, test on EBL disease has been conducted by BPPV in all Indonesian territory and other treatment is by using tight quarantine. The most used diagnosis as according to EBL expert meeting in Copenhage Germany 1997 is by using *Agar Gel Immunodifusi* through *Ouchterlony*

test. The test results in white precipitation lines as result of antigen band, specific antibody in *outchterlony agar*. The positive result of white lines prejudices the existence of EBL virus (Nossal, 1998; Ishino, et al, 2000; Nakajima, et al, 2000; OIE, 2003).

Antigen KIT from import product, beside the price is high and needs long time period to arrive in target places, moreover if the place is far from anywhere, sometimes the ox has been death before antigen KIT hasn't been arrived yet.

Endemic event can happens because of low awareness of farmers to report the epidemic of death ox to related institutes; the existence of transmission can cause negative fausta, it is very dangerous because whether the ox has been positive EBL is never known, finally it can cause high infection and epidemic explosion because this disease has characteristic of endemic beside that limited fund to detect the existence of EBL disease thoroughly in all suspected territory. Based on the description above, we have been motivated to research the production of sub unit vaccine candidate of *protein Egp 51* local isolate EBL virus.

The production of *protein Egp 51* local isolate EBL virus as candidate of the vaccine starts from preparation grower medium of EBL virus, that is, *Cell Line Ovine Lung (OL)* obtained from lung of young sheep with the age of 4 weeks which we obtain from RPH Pegirian Surabaya. On the grower medium, *Cell OL* is inoculated with EBL virus from next pasase (pasase 16) which comes from local isolate EBL virus in Lembang, West Java, which previously have been developed on pasase 15 from BPMSOH Gunung Sindur, Bogor.

EBL virus is adapted up to pasase 29 while titrated to get virus which have adapted in grower medium of Cell OL and has high titer $10^{7.8}$ $TCID_{50}$ as candidate of *protein Egp 51* sub unit vaccine. Koch postulate test is conducted to determine whether EBL virus has different feature with natural virus after conducting pasase up to 29 times on Cell OL. To prove that EBL virus is exist AGID and invitro tests is conducted on grower medium of Cell OL to result in *cytophatogenic effect (CPE)*.

The other test how to know about the EBL virus, I finished research KIT diagnostic antigen of EBL local isolated similar with KIT diagnostic antigen of EBL a Canada produced.

Result of pasase EBL virus with high titer $10^{7.8}$ $TCID_{50}$ sonication is conducted with sonicator to get *envelope (E)* of EBL virus which has *protein Egp 51*. Characterization with *SDS-PAGE*, we get several kind of EBL protein such as P15 kDa, 24 kDa, 30 kDa, and *envelope glycoprotein* gp51 kDa. To get *protein Egp 51*, the next step is elution then continued with *immunoblotting* through *Western Blott*. This will result in one *band* pure of *protein Egp 51*. Production of polyclonal antibody by injecting EBL virus to rabbit plus *Freund Adjuvant Incomplete (FAI)* and *Freund Adjuvant Complete (FAC)* result in polyclonal antibody which used to *immunosrespon* invitro test consists of *Haemaglutinastion* test, obstacle test of *haemaglutination*, *immunocytokimia* test, and *Agar Gel Immunodifusi* test. The next step to know immunogenic power and protective, invivo test is conducted by using male rabbit as much as 50 and weight of 4 kg. Invivo test consists of group I as control group which isn't given *protein Egp 51* but is challenged with savage virus with titer $10^{7.8}$ $TCID_{50}$; group IIa, IIb is group which is given vaccine candidate of *protein Egp 51* and is boostered one time for group IIa (P2) and two times for group

IIb (P3), each on second week, while group IIc is group which is challenged on third week with savage virus with titer $10^{7.8}$ $TCID_{50}$. Group II is group which isn't vaccinated and isn't challenged. From the result, we can see that on the group which is not given sub unit vaccine candidate of *protein Egp 51* the rabbits are death and arise bumps in all *lymphoglandulae*, viscera tool, stomach swelling, hearth swelling and *hepatomegali* is exist. If the bumps is sliced then it release pus which constitutes of mass of oncogenic EBL tumor on group IIa, IIb, and IIc, all rabbits are alive. After conducting examination of blood, we can see the increasing of lymphocyte on group which isn't given *protein Egp 51* and *isn't challenged.*

From this research, we can conclude that *protein haemagglutinin Egp 51* is local isolate EBL virus and has characteristic of *immunogenic* and *protective* which can be used as sub unit vaccine candidate.

ABSTRACT

Isolation And Characterization Of Immunogenic Protein *Egp 51* Of Local Isolate EBL Virus As Sub Unit Vaccine Candidate

Experimental Laboratories Research

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It has been found that *protein Egp 51* virus *Enzootic Bovine Leucosis* (EBL) local (field) isolate is suitable for a candidate of protein sub-unit vaccine. *Protein Egp 51* is obtained from several research series, starts from research of making grower medium of virus EBL, made from foetus's lung of sheep with age of 4 week, obtained from Animal Slaughtering House of Pegirian, Surabaya. From this grower medium, we result in *cell line* cell after being grown to pasase 58 in *maintenance cell* medium which contains *fetal calf serum* (FSC) 5%. *Cell line* of foetus's lung of young sheep or *ovine lung* (OL) is cultivated with EBL virus which comes from field (local), obtained from BPMSOH Gunung Sindur Bogor. The EBL virus is developed and adapted until pasase 29 then ditritated and calculated with Karber method, and finally result in titer virus $10^{7.8}$ TCID on pasase 29. To make sure the existence of EBL virus AGID test is conducted and the existence of *cytopahtogenic effect* on the result of inoculation in OL culture breeder.

Virus with high titer $10^{7.8}$ $TCID_{50}$ is purified by *sucrose gradient* with gradual concentration from 20%, 40%, 50%, 60% and 80%. After that virus suspension is poured as much as 4 ml and then centrifuged with velocity 35,000 rpm (Ultra Centrifuge L8-60 M, Beckmann) on the temperature of 4^0 C as long as 2 hours, with opposite light we can see white ring which constitute of EBL virus particle comes from purification as much as 1.22 gr/cm^3/4 ml.

Envelope (E) of EBL virus is obtained from purification of EBL virus, and then sonication is conducted with sonicator. After that characterization is conducted through *SDS-PAGE Electrophoresis*. In this way, several protein such as 15 kDa, 24 kDa, 51 kDa, 75 kDa, 93 kDa, and 102 kDa is found. The next step is to conduct elution, cutting in order to result in *SDS-PAGE* on 51 kDa, then characterized with *immunoblotting* by using *Western Blott*, found one *band protein Egp 51*. It means that *protein Egp 51* has been purified. The next step is to conduct *invitro* test by using poliklonal antibody which resulted from injection of EBL virus plus *Freund Adjuvant Incomplete (FAI)* and *Freund Adjuvant Complete (FAC)* on rabbit. The *Invitro* test we conduct is consist of hemaglutination test, obstacle test of hemaglutination, immuno histochemical test, neutralization serum test. After that, we conduct *invitro* test on rabbit groups which classified into group I or control group: rabbit is challenged with savage virus titer $10^{7.8}$ $TCID_{50}$ without giving *protein Egp 51*; group IIa and group IIb is group with *protein Egp 51* then booster one time (P2) for group IIa and booster two times (P3) for group IIb, while group C is given *protein Egp 51* on third week and challenged on savage virus $10^{7.8}$.

From the result of research we found that *protein haemagglutinin Egp51* of local isolate EBL virus has characteristic of immunogenic and protective which can be used as candidate of protein sub unit vaccine on EBL disease.

Keywords: *Cell Line OL, local isolate* EBL *virus, characterization of protein Egp 51 kDa, Purification, Sonication, Immunoblotting, Western Blott, Challenge, Immunogenic, Protective.*