RINGKASAN

DETEKSI PROTEIN CATHEPSIN-L UNTUK PENGEMBANGAN DIAGNOSIS DISTOMATOSIS DENGAN TEKNIK ELISA (Sri Mumpuni Sosiawati, Sri Subekti B.S., dan Kusnoto, 35 halaman)

Penggunaan antigen *crude protein* untuk diagnosis distomatosis tidaklah spesifik, karena antigen ini terdiri dari berbagai macam protein sehingga dikenali pula oleh antibodi terhadap cacing lain. Oleh karena itu perlu dilakukan isolasi dan karakterisasi terhadap protein spesifik, agar diperoleh protein murni dengan sensitivitas dan spesifisitas yang tinggi. Penelitian ini mencoba untuk mengisolasi protein *cathepsin*-L (CatL) yang diperkirakan massa molekul relatif (MR) 27-28 kDa dari protein ES *Fasciola spp* kemudian dilakukan karakterisasi protein. Adanya ikatan antara antigen CatL dengan antibodi anti-CatL merupakan dasar dari penelitian ini.

Penelitian ini secara umum bertujuan memperoleh protein antigenik dengan sensitivitas dan spesifisitas tinggi yang dapat dibakukan untuk pembuatan *kit diagnostik* untuk diagnosis distomatosis melalui pemeriksaan serum darah penderita.

Pada penelitian ini dilakukan isolasi dan karakterisasi protein yang berasal dari protein *excretory-secretory* (ES) dari cacing *Fasciola spp* dengan cara mereaksikan protein murni dengan antibodi poliklonal. Pada tahap pertama *Fasciola spp* dewasa diinkubasikan dengan medium RPMI untuk memperoleh protein ES, selanjutnya protein ES diidentifikasi melalui SDS-PAGE dengan pewarnaan silver. Kedua, protein ditransfer ke membran nitroselulose menggunakan *transblotter* dan direaksikan dengan antibodi poliklonal anti-*Fasciola spp* yang kemudian divisualisasikan melalui konjugat *goat-anti mouse* dan pewarnaan *Western blue*. Ketiga, menentukan fraksi protein dilakukan berdasarkan pada nilai MR dan kemudian dilakukan isolasi protein dengan preparatif gel elektroforesis. Keempat, uji antigenesitas, sensitivitas dan spesifisitas terhadap protein CatL yang berhasil diisolasi pada tahap ketiga maupun protein ES yang diisolasi pada tahap pertama.

Hasil penelitian menunjukkan bahwa: 1) Telah diketahui 16 macam fraksi protein ES *Fasciola spp*, yaitu pada MR 130, 108, 91, 74, 58, 52, 45, 40, 35, 32, 28, 27, 25, 18, 15, 8 kDa; 2) Telah berhasil diidentifikasi protein menujukkan duabelas ikatan protein dengan karakter yang berbeda, yaitu protein pada BM 130, 108, 58, 45, 40, 35, 28, 27,

25, 18, 15, dan 8 kDa; 3) Telah berhasil diisolasi protein *cathepsin*-L (CatL) dari ES *Fasciola spp*, yaitu pada BM 27-28 kDa; dan 4) Sebagai bahan uji, protein CatL dengan nilai sensitivitas 63,6% dan nilai spesifisitas 87,5% lebih baik dibanding protein ES dengan sensitifitas 100% tetapi spesifisitasnya 0%.

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SUMMARY

PROTEIN CATHEPSIN-L DETECTION FOR DEVELOPING OF DISTOMATOSIS DIAGNOSTIC BY ELISA TECHNIQUE (Sri Mumpuni Sosiawati, Sri Subekti B.S., and Kusnoto, 35 pp)

The use of crude protein antigen to diagnose of distomatosis is actually not specific, because this type of antigen consists of various proteins that has been recognized by other worms. Therefore, it is necessary to isolate and characterize a specific protein in order to produce a pure protein which has high sensitivity and specivicity for the diagnostic aid of distomatosis. In this research cathepsin-L (CatL) protein with relative molecular mass (MR) 27-28 kDa originated from ES *Fasciola spp* protein was characterized based on the binding amongst CatL and anti CatL antibodies.

This research was aimed to find out antigenic protein with high sensitivity and specivicity for producing a diagnostic kit of distomatosis through blood test of patients.

Isolation and characterization of protein originated from excretory-secretory (ES) of Fasciola spp were performed by the reaction of pure protein and polyclonal antibodies. In the first step mature of *Fasciola spp* were incubated in RPMI medium for producing ES protein, this later protein was identified by SDS-PAGE with silver stain. The second step, this protein was transferred to nitrocellulose membrane by using transblotter and reacted with polyclonal antibodies anti-*Fasciola spp* then was visualized through goat-anti mouse conjugate and western blue staining. The third step, the determination of protein fraction were carried out based on the result of MR and then this protein was isolated by preparative gel electrophoresis. The fourth step, tests of antigenicity, sensitivity and specificity for CatL protein which has been isolated in the third step and these procedure has also were carried out for protein ES which has been isolated in the first step.

The results of the research showed: 1). It has been found 16 types of protein fractions ES Fasciola spp, ie. in the MR 130, 108, 91, 74, 58, 52, 45, 40, 35, 32, 28, 27, 25, 18, 15, 8 kDa; 2). It has been identified protein which showed 12 protein bound with different characters, that were protein in the molecular weight (MW) 130, 108, 58, 45, 40,

35, 26, 27, 25, 18, 15 and 8 kDa; 3). It has been isolted cathepsin-L (CatL) protein from ES Fasciola spp, that was at the MW 27-28 kDa; and 4). As a material for diagnostic test, protein CatL with the points of sensitivity 63.6% and spesificity 87.5% was better than protein ES with the points of sensitivity 100% but its sensitivity was 0%.

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