

Profile of Antigen Excretory-Secretory *Schistosoma Japonicum* in the Development of Elisa Method to Detect Schistosomiasis in Indonesia

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ABSTRACT

The development of ELISA method to detect patients with schistosomiasis in Indonesia is a detection of the antigens that are derived from the peripheral vessels in the blood serum. This research is conducted in the Napu Plateau of Poso Regency, Central Sulawesi, Indonesia. The goal is to develop ELISA method with polyclonal antibodies to detect antigenic-excretory antigen of *S. japonicum* in patients with schistosomiasis. Activities in the study include activities in the field namely the isolation of worm *S. japonicum* and activities in laboratories such as production, characterization and purification by Bradford methods as well as electrophoresis test to see the protein Antigen profile. The results of the study of the 30 worm *Schistosoma* collected from 5 rats suffered schistosomiasis, obtained as much as 42.5 ml with a protein concentration of 1.351 mg/ml, and had two patterns of polypeptide tape with a molecular weight range of 20 and 39 Dalton kilo (kDa). The conclusion of the resulting Antigen ES *S. japonicum* profile can induce a good polyclonal antibody in the development of ELISA method to detect the AgES in the serum of schistosomiasis.

Keywords: Antigen Excretory-Secretory, *Schistosoma japonicum*, ELISA

1. INTRODUCTION

Schistosomiasis is one of the neglected tropical diseases [NTD] endemic in 78 countries and infects more than 229 million peoples in tropical and subtropical regions [1]. There are 2 major forms of schistosomiasis, intestinal and urogenital, caused by 5 main species of blood fluke. The three main species causing human disease are *Schistosoma japonicum*, *Schistosoma mansoni* and *Schistosoma haematobium* [2]. Among them, *S. japonicum* is prevalent mainly in China, the Philippines and some areas of Indonesia [3]. Other research says there are seven species such as schistosomiasis in humans caused by *Schistosoma mansoni*, *S. haematobium*, *S. japonicum*, *S. intercalatum*, *S. mekongi*, *S. malayensis*, and *S. guineensis* [4]. Schistosomiasis ranks second next to malaria from parasitic infection in terms of socioeconomic and health impact in tropics and subtropics [5]. Schistosome parasites lay up to a thousand eggs per day inside the veins of their mammalian hosts. The immature eggs deposited by females against endothelia of venules will embryonate

within days. Approximately 30% of the eggs will migrate to the lumen of the intestine *japonicum* continue the parasite life-cycle. Many eggs, however, are trapped in the liver and intestine causing the main pathology associated with *Schistosoma mansoni* and *japonicum*, the liver granulomatous response [6]. In Indonesia, schistosomiasis on humans and animals are caused by worm species *Schistosoma japonicum*, and only found in Central Sulawesi in Napu, Bada, and Lindu highlands [7] with an intermediary host *Oncomelania hupensis lindoensis* (*O. hupensis lindoensis*) [8]. Control program implemented Until now, we haven't been able to press numbers schistosomiasis infection, due to reinfection from various reservoirs including rats, livestock, wild animals, and even the people themselves as source of infection [9]. The development of immunological and molecular techniques is a new alternative in early diagnosis of various pathogens [10].

Molecular Xenomonitoring is a DNA-based method that has been developed to monitor the transmission of several vector-borne diseases,

including trypanosomiasis [11], filariasis and malaria [12], helminthiases [13], and fascioliasis [14], including to some extent schistosomiasis [15]. This study developed an immunological technique of detection of schistosomiasis based on the use of antibodies with targeted antigen parasites sought.

2. MATERIALS AND METHODS

Based on the activities, we divided the existing work into two categories.

2.1 Activities in the Field

The *Schistosoma* worms used came from wild mice infected with schistosomiasis.

2.1.1 *Schistosoma japonicum* Worm Collection

Schistosoma Japonicum worms collected from rats caught in a positive focus by using life trap. The trap used was made of wrought iron. The caught rats were collected and taken to the schistosomiasis laboratory. To remove worms, surgery was performed in rats. In surgery especially those observed were the gastrointestinal tract and intestinal fork of the portal vein and mesenteric vein. The collected adult worm was washed three times with a physiological NaCl solution to remove the remaining blood that sticks to the worm. The physical condition of the worm was observed with a stereo microscope or a loop, the still alive and intact worm collected in containers that contain physiological NaCl. A life-trap method based on the schistosomiasis control manual [16].

2.1.2. Activities in the Laboratory

Isolation and production of ES antigens in this study that have been modified [17]. Measurement of protein concentration using the Bradford method [18]. Determine the pattern of protein bands with *Sodium* Deodecil Sulphate-Poly Acrylamide Gel Electrophoresis (SDS-PAGE) [19].

2.1.2.1 Isolation and Production of ES Antigens

Isolation and production of ES antigen in this study has been modified [17]. ES Antigen was a result of the metabolism of *S. japonicum* worm used to produce the origin of goat IgG antibodies. In this study antigens were produced with: The collected and surviving worms were transferred into the petri 50 ml containing a solution of phosphate buffer saline (PBS). The clean worm was moved into a container containing the 1640 RPMI media containing antibiotics that were temperature 37 °C for 15 minutes. Then all surviving worms were moved into a solution of RPMI 400 (a new one in a well microplate and incubated at 37 °C for 4 hours) 2 worm in 400 (L

RPMI). After incubation completed solution containing ES antigen collected in one container to be stored at a temperature of -21 °C before use.

2.1.2.2. Characterization of ESA *S. japonicum*.

ES antigens were produced in centrifugation at 4°C at a speed of 2500x g for 10 minutes. Supernatants and pellets were separated, and Bradford tests were conducted to determine the concentration of ESA produced by the BSA solution standard (Bovine Serum Albumin). If the concentration was lower than 1,000 mg/ml then it was best to do so. Bending is carried out with, supernatant and pellet precipitated with ammonium sulphate 40% (w/v) for 24 hours at a temperature of 4 °C while being stirrer. Pellet was dissolved with PBS comparison 1:4 before being deposited with ammonium sulphate. Precipitated supernatants and pellets were then dialysis using cellulose membranes in a 500 ml PBS solution for 4 hours, where PBS was replaced every hour. Purified supernatants and pellet solutions were stored at a temperature of -200 °C until the moment of use. Purification and measurement of protein concentrations was measured by Bradford test [18]. The resulting concentration of protein ESA was 1,351 mg/ml. The ES *S. japonicum* Antigen has two polypeptide ribbon patterns, with a molecular weight range of 20 dan 39 kDa. An ES protein antigen profile was produced through electrophoresis test using Coomassie Brilliant Blue staining, as in the following image [19]. The protein profile was seen in the gel so that obtained from the SDS-PAGE test the molecular weight was calculated using a linear regression test, with a unit of kDa.

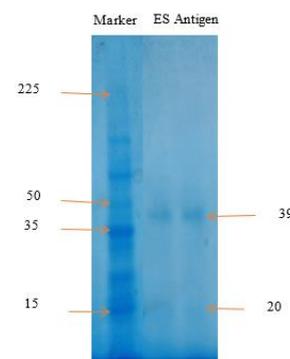


Figure 1. The profile of *Shcistosoma japonicum* ES antigen protein

2.2. Our Contribution

This paper presents several activities based on the development of diagnostic methods to detect schistosomiasis sufferers in Indonesia, which has been using only the standard method that is with Kato Kats. This method is still the standard gold method in the

examination of worm infection, but in the event of a low case (prevalence below zero) the application of this method is no longer relevant, so a faster, precise and accurate method of discovery is required. Immuno diagnosed development detects schistosomiasis early on has been done and commercialized such as antibody detection but has not been used in Indonesia. In 2017, the case has been suppressed to below zero. So that in screening activities needs a faster, precise and accurate method. Currently the development of methods using urine is still ongoing but has not shown maximum results. Based on this, the development of ESA *S. japonicum* detection methods in schistosomiasis sufferers may provide input for further consideration and development, as a method of detection of schistosomiasis.

2.3. Paper Structure

The rest of the paper is organized as follows. Section 2 shows how ESA produced in this study can be used in the manufacture of antibody in goats. Then carried out the manufacture of serums from schistosomiasis sufferers and serums from people who are free of schistosomiasis. Furthermore, the development of methods by optimizing Enzyme Linked Immunosorbent Assay (ELISA) model to get conformation model that can detect schistosomiasis sufferers. The last is to conclude from the results of this study, and the direction for future research.

3. RESULTS AND DISCUSSION

Table 1. Formation of polyclonal antibodies in goat treatment based on AGPT test results

ESA	Antibody Formation in weeks														
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	
	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+

The antibodies that have been formed are then purified using a purification antibody kit (Montage®). The resulting concentration of polyclonal antibodies is 0.931 mg/ml. The concentration of antibodies is measured using the Bradford method [18]. The formation of antibodies may vary depending on the immunogenicity, shape, stability of the stimulant, animal species, injection route, as well as the sensitivity of the test used to detect antibodies [21]. The lowest concentration of antibodies detected using the AGPT test is 30 µg/ml [22], while according to Kuby the minimum antibodies in the serum that can be detected by the AGPT test are 20 µg/mL [23].

3.1.3. ELISA Test Optimization

Based on the results of test optimization on ages layer obtained igg multiple value 1.2-1.5 times

3.1. Antibody Production and Characterization

3.1.1. Animal Preparation

The animals used in this study were two goats, one as a control and one as a treatment. Both goats were kept in cages that have no contact with the soil. A month before the treatment of goats was treated with the administration of worms, scabies and vitamin B to free goats from worm and skin diseases during the study. The feed given to goats is in the form of green grass that has been floated first, the purpose so that the metacercaria that is on the grass. Before treatment is carried out fecal examination on goats to ensure goats are free from worm infection.

3.1.2. Production of Polyclonal Antibodies

The first injection using 150 (g/tail antigen ES without being added adjuvant with intra vein route (I.V). The second injection route is under the skin (S.C) using ES antigen added adjuvant complete comparison 1:1. After an interval of two weeks carried out the third injection with the administration of antigen ES added adjuvant in complete each comparison 1:1. In the third week the blood is taken through the veins for qualitative examination by the agar gel precipitation test (AGPT) method. Goat origin polyclonal antibodies formed after 12 weeks, immunized with ESA *S. japonicum* [20].

negative sample absorption value, then performed optimization on IgG with concentrations of 2 and 1 µg /mL. The IgG coating on this treatment is combined with a positive serum of schistosomiasis sufferers with 20 times dilution. Results were obtained at a concentration of igg 2 µg/mL, i.e. with multiples of 0.97-1.5 times the negative sample value, and at IgG concentrations of 1 µg/mL, multiples of 1.1-2 times the negative sample value [24]. Visually it also appears to differ in color between IgG concentrations of 2 µg/mL and 1 µg/mL in ages binding in serum schistosomiasis sufferers. At a concentration of 1 µg/mL, blue visualization is more concentrated than ESA binding at a concentration of 2 µg/mL IgG coating.

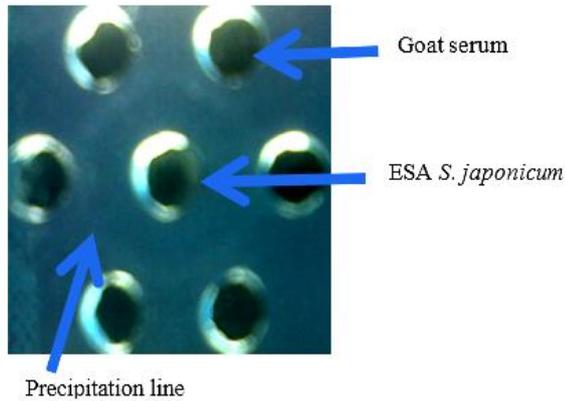


Figure 2. AGPT ESA test results with goat serum

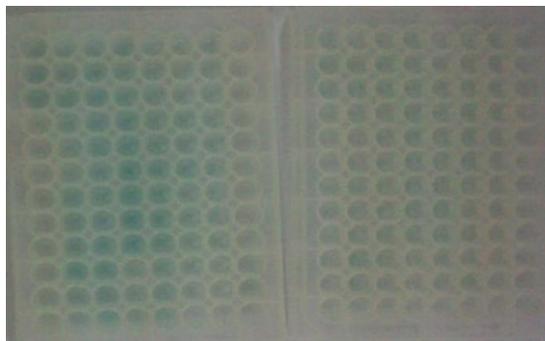


Figure 3. Visual color difference of IgG coating concentration of 1 -2 µg/mL.

The smaller the concentration of IgG used in the binding of AgES *S. japonicum* in the blood of the sufferer indicates the more sensitive the model developed [25].

Optimization of ELISA tests to determine the positive and negative absorption values of schistosomiasis, carried out by comparing positive serum and negative serum schistosomiasis with PBS absorption values. The absorbance value obtained from IgG optimization of 1 µg/mL in negative serums, ranges from 0.263 to 0.32, and the absorbance value in positive serums ranges from 0.248-0.403 with a cut off of 0.307. At a concentration of 1 µg/mL there is about 19% of positive serum samples that are below the cut off value in this case the negative zone, while for negative serums 100% below the cut off value [24].

4. CONCLUSION

Antigen ES *S. japonicum* from rat with the 20-39 KDa protein profile was capable of inducing antibodies in goats. In the development of schistosomiasis

diagnosis method, the resulting antibody concentration of 1 µg/mL as capture on ELISA examination to detect the ESA in the serum of patients with schistosomiasis.

AUTHORS' CONTRIBUTIONS

SS, MAN and MM contribution to collect the sample and the laboratory work in produce of antibody and characterization. SM and FS contributed to review and analysis result of this study.

ACKNOWLEDGMENTS

This work is supported by the Health Research and Development Agency of the Ministry of Health R.I; Donggala Health Research and Development Center (DIPA 2012); Laboratory of Napu schistosomiasis (Poso District, Central Sulawesi); Medical Microbiology Laboratory of Bogor Agricultural University.

REFERENCES

- [1] WHO, Schistosomiasis [Internet], (2020) available from: <https://www.who.int/news-room/fact-sheets/detail/schistosomiasis>.
- [2] T. Meningher, G. Lerman, N. Regev-Ridzki, D. Gold, I.Z. Ben-Dov, Y. Sidi, D. Avni, E. Schwartz, Schistosomal miRNAs isolated from extracellular vesicles in sera of infected patients; a new tool for diagnosis and follow-up of human schistosomiasis, *J. Infect. Dis.* 215(3) (2016) 78-86. DOI: 10.1093/infdis/jiw539.
- [3] A.G.P. Ross, R.M. Olveda, L. Acosta, D.A. Harn, D. Chy, Y. Li, D.J. Gray, C.A. Gordon, D.P. McManus, G.M. Williams, Road to the elimination of schistosomiasis from Asia: The journey is far from over. *Microbes Infect.* 15(8) (2013) 58-65. DOI: 10.1016/j.micinf.2013.07.010
- [4] T. Hailegebriel, E. Nibret, A. Munshea, Prevalence of *Schistosoma mansoni* and *S. haematobium* in snail intermediate hosts in Africa: A systematic review and meta-analysis, *J. Trop. Med.* 2020 (2020) 8850840. DOI: <https://doi.org/10.1155/2020/8850840>
- [5] M.T. Inobaya, R.M. Olveda, T.N. Chau, D.U. Olveda, A.G. Ross, Prevention and control of schistosomiasis: a current perspective, *Res. Rep. Trop. Med.* 5(5) (2014) 65-75.
- [6] C. De Marco Verissimo, J. Potriquet, H. You, D.P. McManus, J. Mulvenna, M.K. Jones, Qualitative and quantitative proteomic analyses of *Schistosoma japonicum* eggs and egg-derived secretory-excretory proteins, *Parasites and Vectors*

- 12(1) (2019), 1-16, DOI: <https://doi.org/10.1186/s13071-019-3403-1>
- [7] A. Samarang, P.P.F Nurwidayati, L.T. Sumolang, T. Lobo, Gunawan, Murni, Kondisi fokus keong perantara schistosomiasis oncomelania hupensis Indoensis di empat desa daerah integrasi program lintas sektor Sulawesi Tengah. *Balaba* 14(2) (2018) 117-126.
- [8] J. Widjaja, A. Nurwidayati, M. S. Pakawangi, M. Maksud, A. Kurniawan, L. Taruklobo, M. Koraag, M. Syahnuddin, Survei daerah fokus keong hospes perantara Schistosomiasis di Kawasan Taman Nasional Lore Lindu Sulawesi Tengah, *Bull. Penel. Kes.* (2018).
- [9] M. Sudomo, Penyakit parasitik yang kurang diperhatikan di Indonesia, In: Orasi Pengukuhan Profesor Riset Bidang Entomologi dan Moluska, Badan Litbangkes, Jakarta, 2008 pp. 1-61.
- [10] S. Samarang, F. Satrija, S. Murtini, M.A. Nurjana, S. Chadijah, M. Maksud, I. Tolistiawaty, Deteksi antigen ekskretori-sekretori *Schistosoma Japonicum* dengan metode elisa pada penderita Schistosomiasis di Napu Sulawesi Tengah, *Media Litbangkes* 25(1) (2015) 65-70. DOI: 10.22435/mpk.v5i1.4098.65-7.
- [11] L.J. Cunningham, J.K. Lingley, L.R. Haines, J.M. Ndung'u, S.J. Torr, E.R. Adams, Illuminating the Prevalence of *Trypanosoma brucei* s.l. in glossina using LAMP as a tool for xenomonitoring, *PLoS Negl. Trop. Dis.* 10(2) (2016) e0004441. DOI: <https://doi.org/10.1371/journal.pntd.0004441>
- [12] D.A.N Cook, N. Pilotte, C. Minetti, S.A. Williams, L. Reimer, A superhydrophobic cone to facilitate the xenomonitoring of filarial parasites, malaria, and trypanosomes using mosquito excreta/feces, *Gates Res. Open* 1 (2018) 7. DOI: 10.12688/gatesopenres.12749.2
- [13] C. Minetti, E. La Course, L. Reimer, J. Stothard, Focusing nucleic acid-based molecular diagnostics and xenomonitoring approaches for human helminthiasis amenable to preventive chemotherapy, *Parasitology Open* 2 (2016) E16. DOI:10.1017/pao.2016.13.
- [14] V. Rathinasamy, C. Hosking, L. Tran, J. Kelley, G. Williamson, J. Swan, T. Elliott, G. Rawlin, T. Beddoe, T.W. Spithill, Development of a multiplex quantitative PCR assay for detection and quantification of DNA from *Fasciola hepatica* and the intermediate snail host, *Austropeplea tomentosa*, in water samples, *Vet. Parasitol.* 259 (2018) 17-24. DOI: 10.1016/j.vetpar.2018.06.018
- [15] J. Hamburger, O. Hoffman, H.C. Kariuki, E.M. Muchiri, J.H. Ouma, D.K. Koech, R.F. Sturrock, C.H. King, Large-scale, polymerase chain reaction-based surveillance of *Schistosoma haematobium* DNA in snails from transmission sites in coastal Kenya: A new tool for studying the dynamics of snail infection, *Am. J. Trop. Med. Hyg.* 71(6) (2004) 765-773. DOI: <https://doi.org/10.4269/ajtmh.2004.71.765>
- [16] Pinardi, Hadidjaja, Schistosomiasis di Sulawesi Tengah, Indonesia, 1985 pp. 12.
- [17] S.E. Estuningsih, Diagnosis of *Fasciola gigantica* infection in cattle using capture- ELISA assay for detecting antigen in faeces, *J. Ilmu Ternak Vet.* 11(3) (2006) 229-234.
- [18] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding, *Anal. Biochem.* 72(102) (1976) 248-254. DOI: [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3).
- [19] U.K. Laemli, Cleavage of structural protein during the assembly of the head of bacteriophage T4. *Nature* 227 (1970) 680-685.
- [20] S. Samarang, I. Tolistiawaty, M. Maksud, Antibodi poliklonal anti ES *Schistosoma japonicum* pada kambing (*Capra aegagrus*) bahan deteksi dini pada penderita schistosomiasis, *Jurnal Penyakit Bersumber Binatang* 2(1) (2014) 57-66.
- [21] J.G. Black, *Microbiology: Principles*, John Wiley & Sons Inc., Virginia, 2005 pp. 470-492.
- [22] I. Tizzard, *An introduction to Veterinary Immunology*, 7th ed, Elsevier Ltd., Philadelphia, 2004.
- [23] T.J. Kindt, R.A. Goldsby, B.A. Osborne, J. Kubly, *Immunology*, 6th ed, W.H. Freeman Company, New York, 2007.
- [24] Samarang, M.A. Nurjana, S. Chadijah, M. Maksud, I. Tolistiawaty, Pengembangan metode ELISA untuk mendiagnosis penderita schistosomiasis di Napu Sulawesi Tengah Tahun 2012. *J. Ekol. Kes.* 13(4) (2014) 308-318.
- [25] Z. Yi, X.Z. Zhi, A highly sensitive diagnostic kit for evaluating therapeutic effect in schistosomiasis cases. *PubMed.* 11 (1992): 686.