Protein Concentration Of Excretory/Secretory Released by *Ascaridia galli* Larvae

Darmawi[1], Ummu Balqis[2], Risa Tiuria[3], Maggy T. Suhartono[3], Retno D. Soejoedono[3], Bambang P. Priosoerjanto[3], Fachriyan H. Pasaribu[3], and Muhammad Hambal[4]

[1] *Laboratory of Microbiology. Veterinary Faculty af Syiah Kuala University*
[2] *Laboratory of Pathology. Veterinary Faculty of Syiah Kuala University*
[3] *Graduate School Teaching Staff Bogor Agriculture of University*
[4] *Laboratory of Parasitology. Veterinary Faculty of Syiah Kuala University*

Abstract

Ascaridia *galli* adult worm were collected in the intestine of village chickens from the traditionally market of Bogor. The eggs in uteri of female adult worms were expelled, prepared and colected under stereomicroscope. The eggs were incubated in sterile aquadestilata at room temperature 21 – 30 days to developed embrionated eggs (L_2). A. ***galli*** larvae (L_3) recovered from laying hen intestines 7 days after each oesophagus inoculation with 6000 L_2 were cultured (5 – 10 ml^{-1}) in flasks containing RPMI 1640 media, pH 6.8, without phenol red and suplemented with 100 units ml^{-1} penicillin G, 100 µg ml^{-1} streptomycin, 5 µg ml^{-1} gentamycin, and 0.25 µg ml^{-1} kanamycin. Cultures were incubated at 37^0C in 5% CO_2 and excretorylsecretory product of L_3 released in culture was collected after 3 days. The amount of concentration was determined from the absorbance at 595 nm. The result showed that protein concentration of excretory/secretory product released by L_3 of ***A. galli*** **were 9.83** ppm. The result indicate that the small amount of protein concentration was determined, but it is generally assummed that the protein released by L_3 of A. ***galli*** play an important role in host immune response mechanisms

Keywords: *Ascaridia galli*, ***larvae,*** *excretory/secretory, nematode*

Introduction

About 100 worm species have been recognized in wild and domestic birds in the USA. Nematodes (roundworms) are the most significant in number of species and in economic impact. Of species found in commercial poultry. the common roundworm (*Ascaridia galli*) is by far the most common. The life cycle of A *galli* is simple and direct. Eggs in the droppings become infective in 10-12 days under

optimal conditions. The infective eggs are ingested and hatch in the proventriculus, and the larvae live free in the lumen of the duodenum for the first 9 days They then penetrate the mucosa, causing hemorrhages, return to the lumen by 17-18 days, and reach maturity at 28-30 days (Permin and Nansen 1998).

The mechanisms that underlie tissue invassion by the invasive stages (L_3) of the parasitic nematode A *galli* are poorly understood, but involvement of as yet protein has been suggested. Protein released by L_3 in ES product associated with parasite invasion, which are tightly regulated and parasite specific, are likely to be essential to the success of parasitism. This protein play an important role to stimulate host immune responses. Here, we employed Bradford assays to examine protein concentration in ES product released by L_3 of *A. galli*.

Materials and Methods

Parasite

Female adult worms were obtained from lumen of village chickens in a commercial abattoir in Bogor. The eggs in uteri of female adult worms were expelled, prepared and colected under stereomrcroscope. The eggs were incubated in sterile aquadestilata at room temperature for 21- 30 days developed embrionated eggs (L_2) (Tiuria *et al*., 2003).

A. galli L_3 were recovered from intestines of 100 heads chickens 7 days after each oesophagus inoculation with 6000 L_2. L_3 recovered in this manner were cultured (5 – 10 ml^{-1}) in flasks containing RPMI 1640 media, pH 6.8, without phenol red and suplemented with 100 units ml^{-1} penicillin G, 100 µg ml^{-1} streptomycin, 5 µg ml^{-1} gentamycin, and 0.25 µg ml^{-1} kanamycin. Cultures were incubated at 37^{0}C in 5% CO_2 and ES product of 4 released in culture was collected after 3 days (Balqis 2004 and Darmawi *et al*. 2006).

Assay of protein concentration

Protein concentration was determined with the Bradford assay. The concentrated samples was stored at -80 °C (Vervelde *et al*. 2005). The protein concentration of ES product was assayed against Coomassie Brilliant Blue. In this study, we prepared a standard calibration curve using bovine serum albumin (BSA). BSA was used as a protein standard. To avoid the interference of glucose present in the RPMI from where the ES product were obtained, 1 ml of ES product was mixed with 100 µl of a 1% sodium deoxycholate solution in 0.1 M NaOH for 5 min on ice. Then, 200 µl of 50% (w/v) of trichloroacetic acid in water were added an

after a further 15-min incubation, the sample was centrifuged at 15,000 rpm for 5 min and the pellet dissolved in distilled deionized water. The dissolved pellet was then used for protein determination (Berasain *et al.* **1997).**

Results

The concentration of released protein during days 1-3 of in *vitro* cultivation (Table 1). When monitored at spectrophotometric, the result showed cultured (5 – 10 L_3 ml^{-1}) the protein concentration was 9.83 ppm.

Table 1. Protein concentration of ES product of L_3 released in culture was collected after 3 days, monitored at 595 nm

Absorbance (sample) 1	Absorbance (sample) 2	Absorbance (mean)	Sample - Blank	Value (a)	Value (b)	Concentration
0.262	0 253	0.2575	0 0255	a 0006	0.0196	9 83

Figure 1. Calibration curve using bovine serum albumin (BSA) to determined protein concentration of ES product released by L_3 of *A. galli* R-Sq = **98.1%; C1 = 0.0196** + **0.00061**7 C2

The regression equation is showed at Figure 1. Protein released by nematodes are recognized as biologically active molecules with important functions relating to host-parasite interaction. This protein are involved in parasite feeding, metabolism, penetration of host tissue barriers, and they are also receiving considerable attention as potentially usefull serodiagnostic or protective antigens (Abe et *al.*, 1992;Cock *et al.*, 1993 and Rhoads *et al.*, 1997). Various authors have indicated that protein played an important role in parasite survival or stimulating the host immune response. Evidence that during transition of L_3 – L. stage larvae of *Ascaris suum*, which are more often associated with molting (Rhoads et *al.*, 1997), have a greater requirement for enzyme to facilitate penetration of host tissues during the extensive migration of the larval stages through the host (Rhoads et *al.* (2001). Proteases secreted by *Ostertagia ostertagi* may be useful target molecules for serodiagnosis designed to detect antibody and vaccination to stimulate host immune response (Cock *et al.*, 1993).

Vervelde et *al.* (2003) invented that a significant increase in antibody levels of lambs immunized with ES. The lambs challenged with 300 L_3 larvae/kg body weight are protected against the parasitic nematode *Haemonchus contortus* and had a reduction in cumulative egg out put of

89%. **Abe** *et al.* (1992) found that protease resistant components in ES **products** of *Stongyloides ratti* adult worms stimulate lymphocytes from infected mice to produce interleukin-3 (IL-3 stimulating activity).

We think *so;* indeed, induction of the poultry immune response against nematode parasites can be controlled by vaccination. Thus, the scientific identification af parasite antigens suitable for inclusion in an anti-nematode vaccine is an important goal. Developing an ideal vaccine against A. *galli*, study fundamental aspects of parasite morphology, biochemistry, and immunology is a must because those aspects strongly determine host-parasite interaction. To date, strategies to identify vaccine antigens in ascaridiosis have relied on ES protein to define antigen.

In this study, parasite protein was observed. The presence of protein in ES product released by L_3 of A. *galli* indicate that the infection process of a number of organisms, including some **nematodes,** depends on protein. The protein are known to play an important role in the pathogenesis of tissue-invading parasites and triggering host immune response mechanisms, including parasitic nematode such as *S. ratti* **(Abe** *et al.* 1992), **Θ.***ostertagi* **(Cock** et *al.* 1993), **A.** *suum* **(Rhoads** et *al.* 2001), and *H. contortus* (Vervelde et *al.* 2003).

The pathogenesis of nematode infection, especially ascaridiosis, is prabably a multistep process (Permin **and** Nansen, 1998) that includes adhesion, degradation, and invasion Adhesion should be the primary step, followed by invasion and degradation of extracellular matrix protein and host cells. Secreted protein of A. *galli* **may** be to facilitate mucosal penetration by the L_3. A. *galli* may likewise utilize the protein to facilitate larval invasion, migration within the host tissue, and modulation of host immune response mechanisms. To increase the value of our finding, additional research will be required to confirm the character of the protein observed at an alkaline pH and at an acidic pH, identified by enzymological studies, and identification of potential vaccine by immunological approaches of ES A. *galli* larval product.

In the effort to get alternative method to handling the poultry from ascaridiosis, it is necessary to know fundamental aspects of A. *galli* morphology, physiology, immunology, and biochimestry. Our early study showed that laying hens infected by A. *galli* could damage mucosal barrier implicated to decrease in villi surface area of intestine (Balqis 2004). We have shown that excretory/secretory (ES) released by A. *galli* adult worm was able to generate intestine rnucosal defence of laying hens

base on prolipheration and hyperplasia of mucosal mast cells (Darmawi and Balqis, 2004). In addition, Balqis (2004) suggested that the ES would be of beneficial in strengthening the host defence mechanisms of intestine mucosa and the laying hens challenged with L_2, did not influence the pathological lesion of duodenum.

Conclusions

In this study, we show that protein present in the ES product of L_3. The secreted protein of A. *galli* showed in present study probably plays an important role in the pathogenesis of infection and other biological processes of the worm. Future study will focus on the purification and characterization of protease(s) and probably important application in defining suitable target antigen for specific serodiagnosis and vaccination to stimulate host immune responses.

Acknowledgements

We wish to thank the Indonesian Ministry of National Education for funding of the work (Grant for 2005 from the Hibah Bersaing: contract number 014/SPPP/PP/DP2M/IV/2004). We thank Ela Nurmalasari, Rahanto Siregar, Indra, and Janiati Karo Karo, for their help; and we thank Sulaeman and Kosasih Department of Parasitology, Veterinary Faculty; Hj Ika Malika Department of Microbiology and Biochimestry, PAU, Bogor Agricutture Institute, for technical assisstance.

References

Abe T, Nawa Y, and Yoshimura. 1992. Protease Resistant Interleukin-3 Stimulating Components in Excretory and Secretory Products from Adult Worms of *Strongyloides ratti*. J. Helminth. 66: 155 – 158

Balqis U. 2004. Microscopical Study of Excretory-Secretory (ES) Product from Ascaridia *galli* Adult Worm, L2, and Their Combination on Duodenum of Laying Hens. J. Med. Vet. 4(1): 261 – 265,

Berasain P, Goni F, McGonigle S, Dowd A, alton JP, Frangione B, and Carmona C. 1997 Proteinases Secreted by *Fasciola* hepatica Degrade Extracelullar Matrix and Basement Membrane Components. J. Parasitrol. 83(1): 1 – 5.

Cock HD. Knox DP. Claerebout E, and Graaf DCD. 1993. Partial Characterization of Proteolytic Enzymes in Different Developmental Stages of *Ostertagia ostertagi* J. Helminthol. 67: 271 – 278.

Darmawi, and Balqis U. 2004. The Effect of Excretory-Secretory (ES) Antigen of *Ascaridia galli* AduR Worm on the Mucosal Mast Cell Response of the Duodenum in Laying Hens J. Med. Vet. 4(1): 255 – 260

Permin A and Nansen JW. 1998. Epidemiology. Diagnosis and Control of Poultry Parasites. *Food* and Agriculture *Organization* of *the United Nations,* Rome.

Rhoads ML, Fetterer RH, and Urban Jr. JF. 1997. Secretion an Aminopeptidase During Transition of Third–to Fourth–Stage Larvae of *Ascaris suum*. J. Parasitol. 83(5): 780 – 784.

Rhoads ML, Fetterer RH, and Urban Jr. JF. 2001. Release of Hyaluronidase During in *vitro* Development of of *Ascaris* suum from the Third to Fourth Larval Stage. Parasitol. Res. 87(9): 693 -697.

Tiuna R, Ridwan Y, and Murtini S. 2003. Study of Bioactive Subtance from *Ascaridia galli* AduR Worm that Stimulate Intestinal Mucosal Defence Mechanism in Chicken for Medical Purpouse. Prod. Toray Sci. Found., Jakarta.

Vervelde L, Bakker N, Kooyman FNJ, Cornelissen AWCA, Bank CMC. Nyame AK, Cummings RD, and Die IV. 2003. Vaccination-induced Protection of Lambs Against the Parasitic Nematode *Haemonchus contortus* Correlates With High IgG Antibody Responses to the LDNF Glycan Antigen. Glycobiol. 13 (11): 795 – 804. http://glycob.oupjournals.org/cgi/content/full/13/11/795 (January 18th 2005).