

# Isolation and Characterization of Excretory/Secretory Antigenic Proteins of Adult *Fasciola gigantica* Lombok Isolate

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## Isolation and Characterization of Excretory/Secretory Antigenic Proteins of Adult *Fasciola gigantica* Lombok Isolate

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### ABSTRACT

The objective of this study was to isolate excretory/secretory (ES) proteins of adult *Fasciola gigantica* local isolate (Lombok) and to determine its antigenic proteins using Western blotting. The ES components were isolated from live adult *F. gigantica* collected from liver of Fasciolosis positive Bali cattle slaughtered at the abattoir. Isolated ES were then characterized using 1-D gel electrophoresis and Western blotting. Gel electrophoresis analysis showed that the ES components of *F. gigantica* Lombok isolate consist of proteins with a molecular weight of 7 to 25 kDa. Western blotting analysis using sera of Fasciolosis positive Bali cattle (n = 2) exhibited several antigenic proteins with a molecular weight of 47.7, 25, 21.4 to 17.9, 16.4 and 10.5 to 8.05 kDa. Amongst the proteins detected, the 25 kDa was sharply reacted in both positive sample. This study suggests that the proteins recognised are an immunologically active component of the ES and antibodies to those proteins were present in naturally infected cattle. The results of this study are expected to support the Fasciolosis control program in cattle through a specific early detection and vaccination.

**Key Words:** Fasciolosis, Bali cattle, 1-D gel electrophoresis, Western blotting, Antigenic proteins

### INTRODUCTION

*Fasciola* sp. release excretory/secretory (ES) components in a significant amount on its host. *In-vitro* biochem studies have showed that ES components play a role in feeding behavior of *Fasciola* sp., detoxification of bile component and evasion of the host immune system (Morphew et al., 2007), and affect the ability of *Fasciola* sp. to survive on host's gall bladder and bile duct.

Several studies have demonstrated that ES antigens isolated from ES components of *Fasciola* sp. could be used as a potential vaccine candidate (Setdavit et al., 2009), and be used for a specific early diagnosis of Fasciolosis in cattle. Kooshan et al. (2010) on their study comparing two types of *Fasciola* antigens, somatic antigens and ES antigens, for diagnosis of Fasciolosis by Elisa technique demonstrated that the ES antigens have a much better response than that of somatic antigens. Nevertheless, cross-reaction with ES components of other trematode, such as *Schistosoma japonicum*, often occurs on assay test resulting in the emergence of false-positive results on examination.

Analysis of 2-D gel electrophoresis indicated that ES components of *Fasciola hepatica* consist of approximately 60 proteins including cathepsin L proteases, superoxide dismutase, thioredoxin peroxidase, Gluthathione S-transferases and fatty acid binding proteins (Jefferies et al., 2001). Among these components, cathepsin L proteases are believed to be the majority component of the ES. Study carried out by Sobhon et al. (1996) for analyzing ES components of *F. gigantica* with 1-D gel electrophoresis showed that dominant constituent proteins of ES components have a molecular weight of 26 to 27, 28, 54, 58, 64 and 66 kDa. Whilst, another study conducted in Iran showed that ES components of *F. gigantica* consisted of dominant proteins with a molecular weight of 15, 16, 20, 24, 33 and 42 kDa (Meshgi et al., 2008). The difference results of above studies indicate that geographical variation and species differences from different host species will affect the constituent components of ES.

To date, there are no published reports on the antigenic proteins of *F. gigantica* local isolate, particularly Lombok isolate, ES components. The purpose of the present study was to characterise the ES antigenic proteins derived from *F. gigantica* Lombok isolate using 1-D gel electrophoresis and Western blotting. The results of this study are expected to provide overview of molecular weight of ES specific antigens of *F. gigantica* local isolate that may be used as a vaccine candidate or diagnostic materials for Fasciolosis in cattle.

## MATERIALS AND METHODS

### Isolation of ES components

An adult *F. gigantica* was collected from liver of infected Bali cattle within 1 h after slaughtered at Banyumulek and Majeluk abattoir. The ES components were prepared according to the method of Awad et al. (2009). Groups of 20 live worms were washed three times with 0.01 M phosphate buffer saline (PBS, pH = 7.2), and were incubated in 10 ml of PBS for 6 to 8 h at 37°C. Worms were then removed and the remaining fluid was centrifuged at 2500 rpm, 4°C for 30 min. Supernatant was collected and filtered using a 0.22 µm sterile filter. The concentration of protein contained in the ES components was determined using Bradford protein assay, and then stored at -20°C.

### 1-D gel electrophoresis

The ES components collected from adult *F. gigantica* were separated using 12% denaturing polyacrylamide. Approximately 20 µg of ES components were then electrophoresed at 100 V for 2 h in a gel apparatus. Ten microliters of Precision plus protein standards dual colour (INtron) were included on each gel as size reference. After electrophoresis, the gel was stained with Coomassie Brilliant Blue for 1 h at room temperature with gentle shaking. The gel was destained in several changes of destaining solution until the background colouration was low.

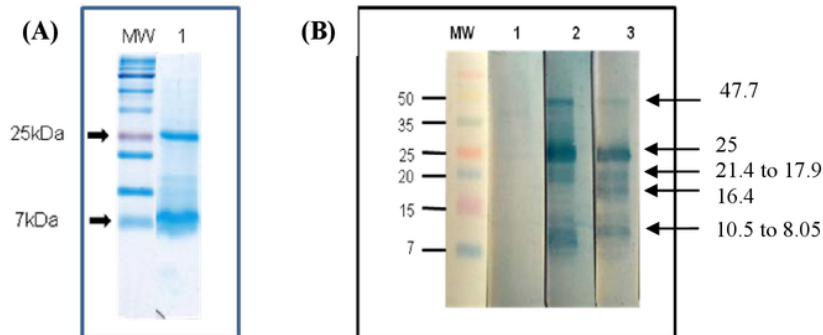
### Western blotting

The transfer of resolved ES components from gel to nitrocellulose membrane was performed for 45 min at 5 V, 0.1 A and 1 W using a Trans-Blot Semi-Dry electrophoretic transfer cell (Bio-Rad). The transfer efficiency was checked by staining the membrane with 0.1% Ponceau-S solution. The membrane was cut into strips and incubated with 5% of skimmed milk in PBS containing 0.05% Tween-20 (PBS-T) at 4°C overnight. After washing 3 times in PBS-T, the membrane containing transferred protein was probed with 5 ml of positive/negative sera on an individual small tray for 1 h at 37°C. The optimum dilution of sera was determined by incubating membrane in different dilutions, and 1:200 dilution was found optimum. Each strip was then washed with PBS-T, and incubated with 5 ml of a diluted goat anti-bovine Ig-HRP (1: 10,000) for 1 h at 37°C. The strips were further incubated in substrate buffer containing 3,3',5,5'-Tetramethylbenzidine (TMB) for 3 min and the colour reaction was stopped by rinsing with distilled water. The molecular weights of proteins were determined by comparing their migration distance against that of a known molecular marker.

## RESULTS AND DISCUSSION

Many reports have indicated that geographical variation may affect the constituent of ES components, and that may suggest a requirement of extensive research on the ES components in different species and different region. Our study on ES component of *F. gigantica* Lombok isolate showed that the isolated ES components composed of protein bands with molecular weight between 7 to 25 kDa (Figure 1A). Study of Meshgi et al. (2008) demonstrated that ES component of *F. gigantica* isolated from cattle has 6 protein bands with sizes ranging from 15 to 42 kDa. Estuningsih et al. (2004) on their study showed that there are three predominant

proteins, 70 to 75 , 35 to 54 and 7 to 21 kDa on SDS - PAGE of *F. gigantica* ES antigens. Although protein bands determined in our study are in the size range of proteins that have been reported by other researchers previously, proteins with a molecular weight greater than 25kDa do not appear on the result of SDS-PAGE analysis.



**Figure 1.** Acrylamide gel (12%) and Western blot of immunoreactive bands of *F. gigantica* Lombok isolate ES antigen. **(A)** SDS-PAGE showing the protein profile of ES components isolated from *F. gigantica* Lombok isolate. Lane MW: standard molecular weight marker; Lane 1: ES components Lombok isolate. **(B)** Western blot using sera obtained from Bali cattle naturally infected with *F. gigantica*. Lane MW: standard molecular weight marker; Lane 1: negative sera; Lane 2: positive sera with EPG = 50; Lane 3: positive sera with EPG = 18.

The result of our SDS-PAGE analysis may suggest that there are differences on protein profile of ES components of *F. gigantica* Lombok isolate or Coomassie Brilliant Blue staining employed in the study is too weak to capture proteins with large molecular weight. Nevertheless, further research is required with a collection of *F. gigantica* from multiple locations. The use of more sensitive staining, such as silver staining, may give a complete profile of the proteins constituent of the ES components.

Identification of proteins of *F. gigantica* that are antigenic and immunogenic is pivotal in an attempt to find a potential candidate for the development of vaccine and specific diagnostic method of Fasciolosis. Estuningsih and Widjajanti (1999) reported the existence of 46 and 47 kDa antigenic proteins isolated from adult, newly emerge juvenile, and 3, 6, 9 weeks of *F. gigantica* on their Western blot. The authors concluded that their finding need to be developed further for specific substances for serological diagnosis of Fasciolosis. Other study showed some antigenic proteins with sizes of 62 to 60, 40, 30, 27, 12 and 10 kDa on Western blot using sera derived from sheep immunized with ES (El Ridi et al., 2007).

Western blotting analysis in this study (Figure 1B) exhibited the presence of antigenic proteins with a molecular weight of 47.7, 25, 21 to 17.9, 16.4 and 10.5 to 8.05 kDa against cattle sera confirmed positive by the presence of eggs in fecal sample. Amongst the polypeptides detected, the 25 kDa was sharply reacted in both positive sample. No visible bands were seen from negative sample on the Western blot.

Western blotting analysis also showed that there is a correlation between the thickness of the protein bands recognised with the degree of infestation indicated by the number of worm eggs per gram faeces (EPG) found in cattle samples (Figure 1B: Lane 2 and Lane 3). Sample with higher number of EPG (50) showed thicker bands than that of lower number of EPG (18).

This study suggests that the proteins recognised are an immunologically active component of the ES and antibodies to those proteins were present in naturally infected cattle. The study also indicates the existence of antigenic proteins with promising diagnostic value in cattle. Further studies, with larger number of samples, would be required to establish the statistical significance of this finding.

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