

Detection of Immunoglobulin Geneheavy Chain Binding Protein in *Eimeria tenella* Collected from Yogyakarta Using One Step Reverse Transcriptase PCR

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ABSTRACT

Coccidiosis is an infection disease caused by *Eimeria tenella* in chicken intestine. The disease causes the economic lost in poultry industry. This disease is very difficult to be controlled for some reasons, *e.g.* the lack of accurate diagnostic tools, expensive vaccination cost, and coccidiost at resistance in *Eimeria*. Immunoglobulin heavy chain binding protein (IHC-BiP) is an immunogenic agent for internal parasite which involves 70 families of *Eimeria* in the heat shock protein. Gene sequence of the binding protein (IHC-BiP) was found in *E. tenella* and *E. maxima* from United Kingdom, however there is no information about this gene in *Eimeria* from Indonesia. This research was aimed to find IHC-BiP gene in four developmental stages of *E. tenella* collected from Yogyakarta. Samples were collected from the field and propagated in 50 chickens. Each chicken was infected with 10.000 *E. tenella* oocysts. The oocyst of *E. tenella* from propagation were then sporulated and prepared for sporocyst and sporozoite stages. Total RNAs were isolated from each stage of *E. tenella* development (unsporulated oocyst, sporulated oocyst, sporocyst, and sporozoite) and the gene was detected by one step reverse transcriptase PCR (RT-PCR). The result showed that the IHC-BiP gene was only found in sporocyst stage of *E. tenella* and did not in other stages. The gene has size of 714 bp. This study suggests that the IHC-BiP protein of *E. tenella* from Yogyakarta is produced during sporulation, especially in sporocyst stage.

Keywords: IHC-BiP, *Eimeria tenella*, reverse transcriptase PCR, Yogyakarta

INTRODUCTION

Eimeria tenella is one parasitic coccidian of seven species of *Eimeria* that causes coccidiosis in chicken. This parasite is the most pathogenic species of *Eimeria* as well as *E. maxima*. Coccidiosis is an economically important disease in poultry industry. Prevention by in-feed medication and treatment contribute a major portion of the losses in addition to mortality, malabsorption, inefficient feed utilization and impaired growth rate in broilers, and a temporary reduction of eggs production in layers. The prevention strategies to control coccidiosis are the usage of anti-coccidial drugs and vaccination. However, there is still needs to develop the alternative control strategy due to rapid emergence of drug resistant field strain of *Eimeria* and high cost associated with development of a new drugs and vaccine (Lillehoj, 2005). Moreover, the lack of accurate diagnostic tools for *Eimeria* also contributes to the difficulty to manage this disease (Subramanian *et al.*, 2008).

Nowadays many recombinant vaccine and molecular diagnoses have been developed. It is cost-effective but the difficulty remains in identification of the antigens or genes which are specific and responsible for eliciting protective immunity. Since *Eimeria* is a parasite with multiple stages of life cycle, it is also important to develop drug or vaccine which targeted on the disruption of the parasite reproduction as earlier as possible (Subramanian *et al.*, 2008). Immunoglobulin heavy chain binding protein (IHC-BiP) is a binding protein which is

included in the heat shock protein 70 (HSP 70) in *E. tenella*. This protein is considered as an immunogenic protein since it plays an important role in parasite invasion and can induce the immune response of the host. The sequence of this protein in *E. tenella* and *E. maxima* shares homology with the chaperone in reticulum endoplasmic (Dunn *et al.*, 1996).

There is still no sufficient information regarding the IHC-BiP gene or protein in *Eimeria* from Indonesia, especially *E. tenella* which is the most abundant species in Indonesia. Therefore, this research aim was to find out in which developmental stages in *E. tenella* collected from Yogyakarta that bears the IHC-BiP gene. The method used in this identification of the gene is by implementation of the one step reverse transcriptase polymerase chain reaction or one step RT-PCR.

MATERIALS AND METHODS

Oocyst sample collection

The *E. tenella* oocysts were isolated from the field (paddy field) in Yogyakarta. These isolated oocysts were propagated in chicken, and the caecum of the chicken was collected after 7 days of incubation. The feces in the caecum were taken and filtered using 325 mesh (45 µm) filter. The filtrates which consisted of unsporulated oocyst were then washed with sterile water three times for 90 minutes of sedimentation process. Some of the unsporulated oocyst suspension was then kept at freezer as unsporulated oocyst sample. The rest of the oocysts were then sporulated using 2.5% potassium dichromate. Sporocyst sample was made from the sporulated oocysts that were broken by glass beads and vortexing. Finally, the sporozoites were created from sporocyst excystation using 2.5% sodium taurocholate and 1.5% trypsin. All the samples were kept in the freezer for further analysis.

mRNA isolation

Prior to mRNA isolation, each of the cells sample were ruptured by glass beads grinding as a modified procedure of Haug *et al.* (2007). After grinding process, the mRNA was isolated using the Total RNA Isolation Kit from SBS Genetech. The total RNA samples were kept at -20°C for further analysis.

Reverse transcriptase PCR

One step reverse transcriptase PCR (One-step RT-PCR) was applied for amplification of total RNA samples. The PCR procedure was based on the protocol of the One-step RT-PCR Kit (SBS Genetech). The modification of procedure during PCR was only to get melting temperature at 48.7°C. The primers for PCR were designed as followed, the forward primer was 3' CGATCTTGGTGGTGGTACC 5' and the reverse primer was 3' AGCGGACTGGTTGTCAGAGT 5'.

Visualization of PCR product

The PCR products were then visualized using 1.5% agarose gel. The electrophoresis was run at 50 Volt electrical powers for about 1 hour. The gel was stained in Good View Nucleic Acid Stain (SBS Genetech) then visualized using UV illuminator.

RESULTS

The four developmental stages of unsporulated oocysts, sporulated oocysts, sporocysts, and sporozoites in *E. tenella* were used in this research. The unsporulated oocyst was the stage of this parasite which came out to the environment together with the feces. This stage then sporulated in the environment to become the infective stage of the sporulated oocyst. In the intestinal lumen of the chicken, the oocyst ruptures and releases the sporocyst which then burst and release sporozoite. Sporozoite is the invasive stage that invades the

intestine epithelial cell to reproduce (Allen and Fetterer, 2002). The morphological feature of these four stages is presented in Figure 1.

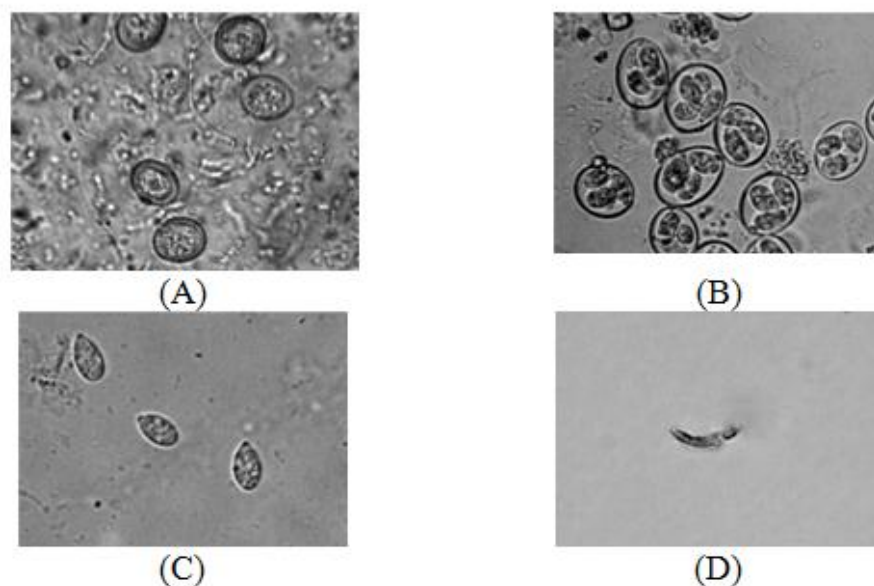


Figure 1. The four developmental stages of *E. tenella* (A. unsporulated oocyst, B. sporulated oocyst, C. sporocyst, and D. Sporozoite)

The detection of IHC-BiP gene in four developmental stages of *E. tenella* collected from Yogyakarta showed that only sporocyst stage which bore the gene. The gene size is predicted in about 714 base pairs (bp). Detection of the IHC-BiP gene was conducted by one step RT-PCR and the result showed in Figure 2.

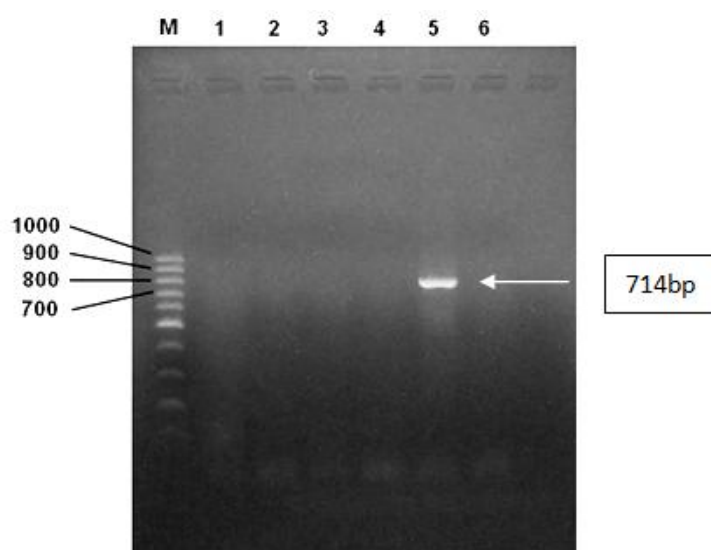


Figure 2. The detection of IHC-BiP gene. The gene was only detected in sporocyst stage (M= marker, 1 and 2= unsporulated oocyst, 3 and 4= sporulated oocyst, 5= sporocyst, and 6= sporozoite)

DISCUSSION

Immunoglobulin heavy chain binding protein (IHC-BiP) is a binding protein found in *E. tenella* and *E. maxima* which is homologue to chaperone in reticulum endoplasma. It is included in the third member of the heat shock protein 70 (HSP70). Many parasites produce the HSP70 protein during their invasion process as a response to an increase of the host's temperature or due to stress from the invasion process (Dunn *et al.*, 1996). In general, HSP70 are among the dominant antigens recognized by the immune system in a large spectrum of parasites. Therefore, it can be assumed that their role in the host-pathogen interaction, this protein is an immunological important (Maresca and Carratu, 1992). In addition, the HSP70 in parasitic protozoa also plays an important role during stages of conversion, infectivity, and virulence. In *Eimeria*, this protein has been found as cellular and molecular events which occur during sporulation phase, the phase that forms the sporocyst and sporozoite (Del Cacho *et al.*, 2008).

Eimeria tenella is the parasitic protozoan which has multiple stages in its life cycle. Many proteins are produced and secreted during its invasion process in the gut epithel (Subramanian *et al.*, 2008). Therefore, it is very important to find the proteins which involve in this process as the materials for vaccine development, drugs, or diagnostic tools so that life cycle of the *E. tenella* can be stopped as earlier as possible.

This research showed that *E. tenella* which isolated from Yogyakarta had the gene that coded the IHC-BiP protein. This gene was only found in sporocyst stadium with the size of about 714 bp. This figures that the gene is only active during sporulation process to produce the protein. The finding is similar to the previous research which was conducted by Del Cacho *et al.* (2008). This research concluded that the IHC-BiP gene found in *E. tenella* collected from Yogyakarta is the gene that encoded the binding protein. This protein isinvolved in HSP70 protein, and therefore the protein is an immunogenic protein.

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