Isolation and Partial Characterization of Proteins from the Mammillary Coat of *Ascaris lumbricoides* Fertilized Egg

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SHORT COMMUNICATION

Abstract

Background and Objectives: The Center for Disease Control (CDC) reported in 2013 that the worldwide prevalence of *Ascaris lumbricoides* infection is 20% of the world's population or a total of 2 billion. Its high prevalence is almost always associated with poor sanitary practices based on several epidemiological studies. Ascaris is usually diagnosed by examining and describing the morphology of the eggs. While it has extensively been described morphologically, few information have been reported about the eggs' biochemistry, especially the macromolecular characteristics of their proteins found on the exterior covering. Unembryonated eggs that are passed out from stool are considered diagnostic stages, which aid in laboratory diagnosis and detection. Morphological examination of eggs is valuable but exploring the protein in their mammillary coat is worthwhile for the development of future diagnostic kits. This study, therefore, was aimed at isolating and partially characterizing the external proteins of the eggs' mammillary coating.
Methodology: *Ascaris lumbricoides'* fertilized corticated eggs from formalinized pooled fecal samples of infected individuals were collected by capillary catch method. Protein coat from the eggs were removed by vortex agitation of the samples in 5% sodium dodecyl sulphate solution followed by ammonium sulfate precipitation. Precipitated proteins were reconstituted in phosphate buffered saline and its molecular weight was determined using SDS-PAGE.
Results and Conclusion: Two distinct protein bands with molecular weights of 75 kilodaltons and 200 kilodaltons were detected. The 75 kDa protein was suspected to be the major constituent of the albuminoid coat while the 200 kDa proteins have not yet been previously identified.

Keywords: *Ascaris lumbrocoides, albuminoid coat, protein characterization, SDS-PAGE*

Introduction

Ascariasis is one of the most prevalent soil-transmitted helminth infections worldwide. According to the World Health Organization, two billion people or 20% of the world's population, which are mostly from the poorest and most deprived countries, are affected by this disease [1]. This infection is widely spread in tropical and subtropical areas, with the greatest numbers occurring in Sub-Saharan Africa, America, China and East Asia, including the Philippines [2]. This disease has been associated with poor hygiene, poor sanitation, and inadequate nutrition [3].

In humans, ascariasis is caused by the nematode *Ascaris lumbricoides*. Infection happens after ingestion of the infective embryonated egg from contaminated food and other fecally contaminated sources. The eggs of this soil-transmitted helminth then hatch into the duodenum. The larvae that are released actively penetrate the intestinal wall and begin its extraintestinal invasion via the hepatic portal route, cardiac vessels, and pulmonary tract. After less than two weeks in the lungs, larvae may reach the trachea and the pharyngeal region until they are swallowed. The parasite reaches maturity in the small intestine. In three-months time after infection, the oviparous female adult *Ascaris lumbricoides* may begin to lay eggs as much as 200,000 per day. It is then that they may be diagnosed through stool examination. Eggs appear fertilized if male worms are present, nonetheless, unfertilized eggs will come out.

Despite the critical role of the Ascaris eggs in infection and laboratory diagnosis, little has been reported on the physico-chemical characteristics of proteins found on their coat. Protein database search yields also very few reports on the coat of the most prevalent soil-transmitted helminth. This study aimed to isolate and partially characterize the proteins present on the coat of *A.lumbricoides* eggs.

This study is an initial approach of the researchers to understand the physical and chemical properties of the proteins on the mammillary coat of the eggs of *Ascaris lumbricoides.* Moreover, since protein in itself is immunogenic, this study may initialize the investigation of its immunogenicity and explore its possible application in diagnostics, prevention, and therapeutics in the future.

Methodology

Ascaris lumbricoides fertilized corticated eggs were collected from pooled fecal samples stored in 10% formalin for less than a month. They were processed through formalin ethyl acetate concentration technique then eggs were taken from sediments and purified by capillary catch method. One millilitre of NSS suspended eggs was placed in a petri dish then viewed under the microscope CX20 Olympus at low power objective. Two-hundred fifty eggs were aspirated using a Pasteur pipet to separate from fecal debris and placed in several Eppendorf tubes. The eggs were washed several times with normal saline solution to further remove the impurities.

Proteins from the egg coat were isolated by agitation in the presence of detergent solution. Two hundred fifty (250) eggs were suspended in 5% sodium dodecyl sulfate solution and were agitated with a vortex for 10 minutes. The protein solution was separated by centrifugation at 15,000 x g for 15 minutes. Anhydrous ammonium sulfate crystals were gradually added until a 35% w/v of solution was achieved to precipitate the proteins. The proteins were reconstituted in phosphate buffered saline and dialyzed. Total proteins were quantified using MicroBradford Total Protein Assay following the Quickstart™ Bradford Total Protein Assay Instruction Manual. Molecular weights of isolated proteins were determined by SDS-PAGE using a broad-range protein molecular weight marker (Promega) as standard.

Results and Discussion

A female *A. lumbricoides* can lay up to 200,000 eggs per day or approximately 60 million eggs throughout its lifetime from a year-round mating with no particular mating season. Egg-laying though is not attributed to the presence of adult male in the intestinal tract. In the absence of the male, the female lays unfertilized eggs instead. From ingestion of the infective embryonated eggs, a larvae attains reproductive maturity after 8 to 12 weeks [5]. Adult worms normally live for a year in the human intestine.

There are two types of Ascaris eggs – fertilized and unfertilized. These two are morphologically distinct when viewed under the microscope in terms of length and egg coat structure. A fertilized *A. lumbricoides* egg is an ovoid shape structure which is approximately 30-40 micrometers by 50-60 micrometers. The larva is encapsulated in a chitinous coat, which is enveloped by a thick mammillary albuminoid protein coat. An unfertilized *A. lumbricoides* egg is more elongated and has an irregular mass of refractile lecithin granules. The eggs do not possess inner lipoidal vitelline membrane but may still have protein mammillations if corticated. Its size approximates 80 – 90 micrometers by 40 – 45 micrometers.

The investigators were able to collect enough number of *A. lumbricoides* from the formalinized pooled fecal samples for protein isolation and characterization. In storage, the eggs maintained the normal rigid structure. Fertilized eggs maintained their integrity showing the distinct tri-layered shells: lipoidal vitelline membrane, glycogen layer, and albuminoid coat. However, some of these fertilized eggs developed and embryonate. Up to 21.79 micrograms of proteins have been isolated from the coat of pooled 250 eggs of *A. lumbricoides.*

As shown in Figure 1, two distinct bands at 75 kilodaltons and 200 kilodaltons were observed when the protein isolates were subjected to SDS-PAGE. Of the two bands, the 75 kilodaltons protein is more likely to belong to the albumin family. Its molecular weight falls within the possible range of albumin from different organisms. Molecular weights of albumin range from 46 kilodaltons (chicken egg ovalbumin) to 79 kilodaltons (horse serum albumin) [7]. The human and bovine serum albumin is closer in terms of molecular weight which is between 60 to 65 kilodaltons. Interestingly, National Center for Biotechnology Information (NCBI) Protein Database searches yield no amino acid sequence report on the proteins present on the *A. lumbricoides* egg coat. Also, the identity of the 200 kilodalton protein has not yet been reported. Studies on other physico-chemical properties of the protein coat of *A. lumbricoides* eggs are still being done by the investigators. Further studies on the amino acid composition, protein structure analysis, and immunogenic sequences are intended to be done.

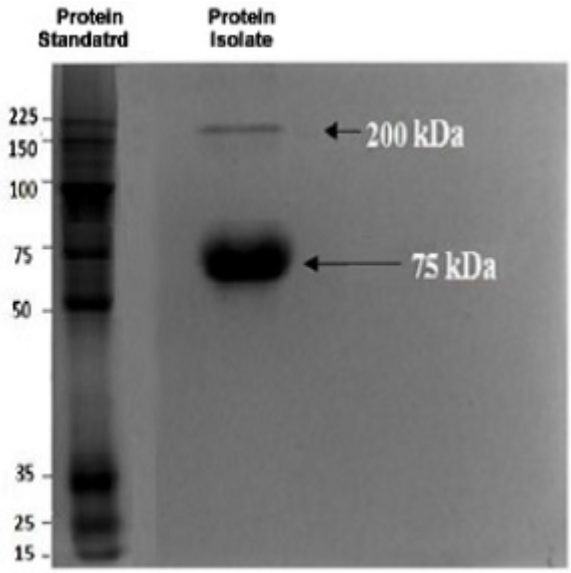

Figure 1. *SDS-PAGE Electrophoretic profile of protein isolates from Ascaris Lumbricoides egg coat reveals two distinct protein bands at approximately 75kDa and 200 kDa*

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