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Isolation of Sheep Sarcocystis 35 kD Protein Fragment by Ion Exchange Chromatography

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Abstract: The genus of sarcocystis parasites have two hosts in their life cycle and are zoonotic. They also have special importance in industrial veterinary. The best way for prevention and control in zoonotic disease is vaccination. This research was planned for sheep sarcocystis antigen isolation by chromatography and a 35 kD antigen was separated by ion exchange chromatography using alkaline (pH=8) CM cellulose resin.

Key words: Sarcocystis, ion exchange chromatography, CM cellulose

INTRODUCTION

The protozoa of sarcocystis genus are obligated intera cellular parasites. The life cycle of these parasites consist of merogony, gamogony and sporogony. Most of them are obligated to have two hosts in their life cycle. A sexual reproduction will happen in the intermediate host artery endothelial cells. Host is always a herbivorous or an omnivorous animal. The last generation of endozoite in striated muscles, nerve tissue and porcine fiber of host heart will form sarcocystis cysts. Metrocysts in premature cyst will develop with consecutive asexual endopolygeny. Mature cysts consist of thousands of cystozoites that will not multiply, this is the last phase of asexual reproduction. The cysts will be ingested by a definitive host (carnivorous animals) and sexual stages of cystozoite will be in epithelial cells of small intestine and oocysts are finally produced. Sporulation of oocyst happens in definitive host lamina properia of small intestine. Final step formation of oocyst is in definitive host. Sarcocystis parasites have special importance in industrial veterinary. These parasites have a negative effect on quality and quantity of meat and wool of animal hosts. Diagnosis of macroscopic sarcocysts in animal will bring about the burial of whole carcass or some parts of it by health staff in the slaughter house. It is estimated that more than one million dollar worth of cow meat is buried annually in the United State of America[1,2]. The best way for prevention of this zoonotic disease is vaccination because control of definitive host is difficult. Domestic animal vaccination is economically very cost effective and the disease can also be controlled in humans. The aim of this project was to separate a single protein of sheep sarcocystis by chromatography to be used in animal sarcocystis detection and vaccination.

MATERIALS AND METHODS

The infected muscles of sheep carcass were collected from slaughter house and transferred to laboratory. The sarcocysts were separated from the infected muscles with scrapple and kept in normal saline for preparing crude antigens. The sarcocysts were freezed and thawed (-22 and 37°C) for 12 times and sonicated. The crude antigen was fragmented by serial dilution of ammonium sulfate solution[3-5] and final separation was done by gel filtration and ion exchange chromatography by alkaline CM cellulose resin (pH=8)[6-8] and was developed on stained commassi brilliant blue 12% SDS-PAGE gel[5].

RESULTS AND DISCUSSION

Some single protein bands were separated by column chromatography and ion exchange chromatography by different pH in positive and negative charges. Finally, a 35 kD protein band was separated by ion exchange chromatography using alkaline CM cellulose resin. Figure 1 shows the protein band on stained commassi brilliant blue SDS-PAGE gel.

Sarcocystis is an important infectious agent in cow, pig and sheep (as intermediate hosts) which can be infected by ingestion of oocyst in feces of dog, wolf, fox,

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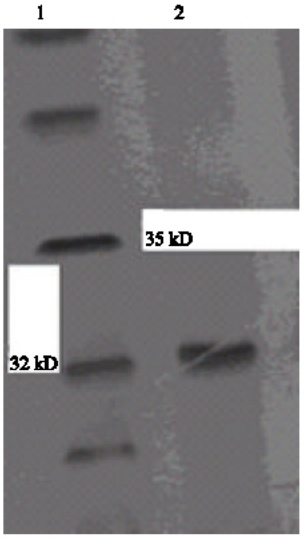

Fig. 1: 12% SDS-PAGE Lane 1: Protein marker, Lane 2: 35 kD purified protein

raccoon and cat (as definitive hosts). Humans can be definitive hosts of sarcocystis hominis (intermediate host is cow) and sarcocystis suihominis (pig is intermediate host). Few hours after ingestion of infected meat by definitive host, the signs of vomiting, anorexia, cramp, gas passing and diarrhea are seen[9-12] which continue for 1 or 2 days. Two weeks later when sporocyst are passing out in feces severe crumps and diarrhea are also seen. The best way to control this infection is to prevent sarcocystosis in domestic animals by vaccination which has already been done[13]. A pure 35 kD protein fraction by ion exchange chromatography from sheep sarcocystis was separated. Other researchers have separated protein fractions from sheep sarcocysts and have used them for detecting parasite in domestic animals. Stride *et al.* and prepared a mixed crude protein from bradyzoites of *S. tenella, S. arieticanis, S. gigante* and *S. muris* and fractionated it by chromato focusing method[6].

Odonoghue *et al.*[14] identified sarcocystis species by isoenzyme electrophoresis methods with selection of sarcocystis cystozoite from macroscopic and microscopic cyst of goat and sheep. Bentz *et al.*[15], Saville *et al.*[16], Blyth *et al.*[17], could detect *S. tenella* in horse by western blot method. Merten *et al.*[18] prepared a recombinant 46 kD protein from *S. tenella* and reported that its antibody can detect *S. tenella* by ELISA. Morsty *et al.*[19] separated sarcocystis cystozoite from infected cow muscles and used it as antigen in ELISA test. Salto *et al.*[20], Savini *et al.*[21] and prepared *S. cruzi* antigen and used it to identify infected cows with sarcocystis via serology.

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