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A Study on Biochemical Composition of the Inner Wall of the Oocyst of *Eimeria necatrix*

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Abstract: A biochemical study was made to elucidate the biochemical composition of the inner wall of oocyst of *Eimeria necatrix* and then compared with cellular component of the oocyst. In addition to total protein, glucose and cholesterol, several electrolyte components have been estimated in the present study. The investigation also revealed high activity of Alanine and Asparate aminotransferases in the inner wall of the oocyst of *Eimeria necatrix*. It seems that the biochemical composition and the enzymes in the inner wall of the oocyst of *Eimeria necatrix* are of significant value for better metabolic processes in *Eimeria* spp. as well as for better understanding of possible drug-parasite interaction.

Key words: Oocyst, *Eimeria necatrix,* alanine aminotransferase, aspartate aminotransferase, electrolyte composition

INTRODUCTION

Coccidiosis is a disease of fowl caused by an obligate microscopic protozoan parasite, which belong to the genus *Eimeria* (Phylum-Apicomplexa). The economic loss from eimeriosis is pooled of mortality and retardation in growth and development of affected poultry and also of reduction in their body weight, egg laying and deterioration of the meat quality (Aliyeva, 1999; Ahmedov *et al*., 2006; Visco, 1975). An important feature of *Eimeria* is the oocyst formation. Eimerian parasites are transmitted from host to host by accidental ingestion of oocysts that contaminate food or water. Oocysts are remarkably hard and able to persist in the environment for prolonged period of time. The soft bodied parasites are safely encapsulated inside a unique structure, the oocyst wall. The oocyst wall is extremely robust. It is resistant to mechanical and chemical damage; breaking oocysts for laboratory studies required prolonged, high-speed vortexing with glass beads and oocysts are routinely cleaned with bleach and stored in the harsh oxidant, potassium dichromate or the mineral acid, sulphuric acid (Dubey *et al*., 1970; Shirley, 1995; Schares *et al*., 2005).

The construction and composition of the two oocyst walls confer the oocyst outstanding biochemical and physiological resistance, thereby making the walls into an effective protective barrier for ensuring the survival of the parasitic organisms in the open. While the outer wall is composed of quinone tanned protein, the inner wall is composed of protein matrix impended with lipid and coated with lipid (Monne and Honig, 1954). These proteins provide the oocyst with great structural stability towards heat or cold. The main objective of the present study was to investigate the comparative biochemical composition and some enzymatic activity between cellular and the inner wall of the oocyst of *Eimeria necatrix*. This information not only is necessary for understanding the parasite metabolism but also for better understanding of possible drug-parasite interaction.

MATERIALS AND METHODS

The oocysts of *Eimeria necatrix* were collected from a poultry farm in Aizawl, Mizoram. The collected oocysts were sporulated with 2.5% potassium dichromate. The sporulated oocysts of the *Eimeria necatrix* were then washed three times using sterile double distilled water to remove potassium dichromate solution. The washed oocysts were then treated with Sodium hypochlorite solution (4% available chlorine) and incubated at 4°C for one hour to remove the outer wall of oocysts. Following incubation, the oocysts were washed several times with sterile double distilled water. The oocysts were then recovered using Zinc sulphate (33.3%) floatation solution by centrifugation at 1500 rpm for 10 min. The oocysts were again washed with sterile double distilled water thrice to remove Zinc sulphate. Finally, the pelleted oocysts were suspended in 100 µl of deionized water, frozen, lyophilized and dry weight was taken. The oocysts were again resuspended in 2 ml of deionized water and sonicated repeatedly at 20 µm at 4°C in 30 sec intervals with periodical checking under compound microscope until 99% of the oocysts were disrupted. The sonicated oocysts suspension was centrifuged at 10,000 x g for 10

min. The supernatant was analyzed for biochemical parameters. The pelleted material was further processed as per the methodology of Stotish *et al.* (1978) with little modification to get purified oocyst wall. Briefly, the pelleted material was dissolved in 1ml of deionized water and centrifuged at 10,000 x g for 10 minutes at 4°C and repeated twice. The pellet was then suspended in 1.0 M sucrose solution and centrifuged at 2500 x g for 15 min. The process was repeated 5 times. The pellet was washed 5 more times by re-suspension in 10 volumes of deionized water at 10,000 x g. The final pellet obtained containing purified oocyst wall was suspended in 1.5 ml of deionized water and analyzed for biochemical parameters.

RESULTS AND DISCUSSION

The cellular and inner wall of the oocyst of *Eimeria necatrix* were studied for their biochemical composition like glucose, cholesterol, total protein, magnesium, phosphorus, calcium and chloride and enzymes, alanine aminotransferase and aspartate aminotransferase. In the present investigation, the outer wall of the oocyst of *Eimeria necatrix* was stripped off by treatment with sodium hypochlorite. The exposure to sodium hypochlorite removes the outer wall of the oocyst but the structure of the inner wall remained unchanged. Figure 1 shows the oocyst of *Eimeria necatrix* with intact inner wall. The same observation was made by Monne and Honig (1954), Nyberg *et al.* (1968), Nyberg and Knapp (1970), Ryley (1973) and Belli *et al.* (2006) in their studies on *E. maxima* and *E. tenella*. Monne and Honig (1954) used a number of destructive treatments that led them to conclude that the outer wall of oocyst contained mainly quinone tanned proteins without lipids. They also noted that the outer layer was stripped off upon exposure to sodium hypochlorite, whereas the structure of the inner wall consisted of a lipid protein matrix; they believed that lipids were bound firmly to proteins, thus protecting the inner wall against disintegration by sodium hypochlorite. Ryley (1973) in his study on *E. tenella* also noted that the outer wall was removed by sodium hypochlorite and found that it contained carbohydrates and proteins, with high proline. The inner wall of the oocyst of *E. necatrix* can be disrupted by repeated sonication at 20 µm at 4°C at 30 sec intervals and it was observed that 99% of the oocysts were disrupted. Figure 2 shows the oocysts of *E. necatrix* after sonication. Stotish *et al.* (1978) also showed that the inner wall of the oocyst can be disrupted by sonication in their study on *Eimeria tenella*. The cellular components and the inner layer of the oocyst were separated by centrifugation at 10,000 x g and the two components were further analyzed for their biochemical parameters. The different biochemical parameters of the cellular component and the inner wall of the oocyst of *Eimeria necatrix* is presented in Table 1. The glucose,

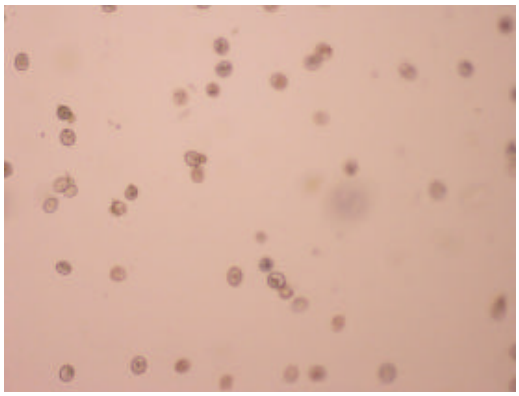

Fig. 1: *Eimeria necatrix* oocysts after sodium hypochlorite treatment (10x)

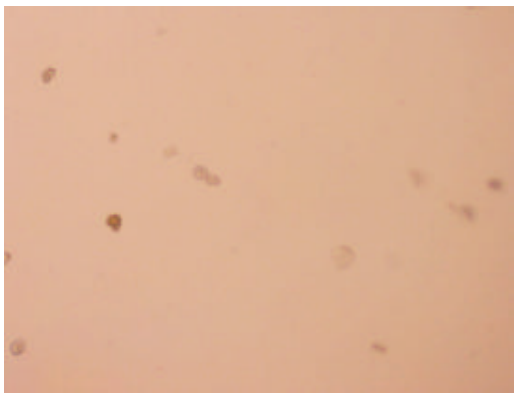

Fig. 2: *Eimeria necatrix* oocysts after sonication (10x)

cholesterol and total protein content of the cellular fraction and the inner wall of the oocyst are respectively 10.47% and 12.42% for glucose, 18.46% and 29.37% for cholesterol and 70.35% and 64.36% for total protein. Since proteins constitute most of the soma and all of the enzymes of protozoans, it is not surprising that the cellular component and inner wall of the oocyst of *Eimeria necatrix* contain a relatively high amount of protein. Glucose content is found little higher in the inner wall compared to cellular component (12.42% vs 10.47%) while the cholesterol content of the inner wall (29.37%) is significantly higher than that of cellular component (18.46%). This observation may be due to the fact that cholesterol is an important component of the cell membranes and a relatively small amount is present in the cellular fraction. The carbohydrate, lipid and total protein content in the inner wall of *E. tenella* was found to be 19% carbohydrate, 14% lipids and 67% protein (Stotish *et al.*, 1978). Therefore, the observation made in the present investigation is almost comparable with the observation made by Stotish *et al.* (1978) for *E. tenella*. A comparison of the glucose and cholesterol content of the oocyst wall of *E. tenella* and *E. maxima*

Table 1: Biochemical composition of the cellular component and the inner layer of the oocyst of *Eimeria necatrix*

Parameters	Method	Cellular component	Inner layer
Glucose	GOD-PAP	10.468%	12.42%
Cholesterol	CHOD-PAP	18.46%	29.37%
Uric acid	Uricase-PAP	2.34%	-
Total protein	Biuret	70.347%	64.36%
Magnesium	Calmagite	0.813%	0.63%
Phosphorus	Phosphomolybdate	0.46%	0.52%
Calcium	O-CPC	0.95%	1.72%
Chloride	Thiocyanate-Hg	14.66 mmol/mg	9.77 mmol/mg

Note: All the estimations were done in a Spectrophotometer, Spectrascan-2600

Table 2: Biochemical composition of glucose and cholesterol of oocyst walls of *Eimeria tenella* and *Eimeria maxima* and inner layer of the oocyst of *Eimeria necatrix*

Parameters	% of metabolites (w/w) *Eimeria tenella* oocyst walls	% of metabolites (w/w) *Eimeria maxima* oocyst walls	% of metabolites (w/w) in the inner wall of the oocyst of *Eimeria necatrix*
Glucose	58.3-61.3%	58.3-61.3%	12.42%
Cholesterol	12.1%	24.2%	29.37%

Table 3: Enzymatic activities in the inner layer of the oocyst of *Eimeria necatrix*

Enzyme	Method	Activities in unit/100 mg
Alanine amino transferase	Reitman and Frankel	800 unit/100 mg of oocyst
Asperate amino transferase	Reitman and Frankel	1300 unit/100 mg of oocyst

reported earlier by Kelly *et al*. (2009) and values estimated in the present investigation in the inner wall of *E. necatrix* is given in Table 2. In addition to proteins, cholesterol, glucose and uric acid, inorganic substances like magnesium, phosphorus, calcium and Chloride have been detected in both cellular components as well as in inner layer of the oocyst of *Eimeria necatrix*. The mineral content of the cellular fraction and inner wall is almost comparable except for calcium and chloride. The amount of calcium and chloride content in cellular and inner wall of the *E. necatrix* are 0.95% and 1.72% and 14.66 mmol/mg and 9.77 mmol/mg respectively. The presence of potassium, calcium, sodium and phosphorus in the avian malaria causing *Plasmodium* (*Plasmodium gallinaceum*) has been reported by Kruszynski (1951). Studies on the electrolyte composition of the oocyst have not been reported till date.

The enzymatic activity of the two aminotransferases *viz*. Alanine aminotransferase and Aspartate aminotransferase of the inner wall of the oocyst of *Eimeria necatrix* is presented in Table 3. The enzyme activities of alanine aminotransferase and aspartate aminotransferase are respectively 800 U/100 mg and 1300 U/100 mg of oocyst. Yagub and Farida (2007) and Williams (1999) also reported enzymatic activities of alanine and aspartate aminotransferases in the oocyst of *Eimeria tenella* and *Eimeria maxima*. The higher activities of the enzyme aspartate aminotransferase than alanine aminotransferase observed in this investigation is in consistent with the findings of Yagub and Farida (2007).

The data obtained by us in the present investigation suggested that by the study of biochemical composition of the oocysts of *Eimeria necatrix* and eimerian enzyme systems the metabolic processes of the parasites and the efficiency of anticoccidial preparations could be estimated and such a study would facilitate goal oriented search for effective and ecologically pure anticoccidial preparations.

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