

DIFFERENTIAL PROTEIN EXPRESSION OF PROGLOTTIDS OF CYCLOPHYLLIDEAN CESTODE, *Raillietina tetragona* (MOLIN, 1858) INFECTING DOMESTIC CHICK, *Gallus gallus*

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Author NA collected the material, executed the plan of work, analysed the results and completed the documentation of manuscript. Author NVK assisted in designing the research problem, supervised the analysis of results, critical reading of manuscript at every level.

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ABSTRACT

Cestodes are endoparasites, and exhibit a great degree of difference in morphology and physiology within the same body. *Raillietina tetragona* infects domestic chick, *Gallus gallus*, the present study was designed to study the regionally specific protein expression in immature, mature and gravid regions by using SDS-PAGE. A total of 14 protein bands were observed ranging in the size from approximately 10,250-95,720 KDa was detected in different proglottids under reducing SDS-PAGE condition, the molecular marker ladder of 170,130,95,72,55,43,34,26,17,10 KDa MW were used. The newly formed protein bands in mature region suggesting rapid reproductive activity, synthesis of proteins required for egg production. The disappeared bands in gravid region indicates the degenerative activities; *i, e* they are not involved in proliferation and scolex formation. The reason for variation is due to differential metabolic activity.

Keywords: *Raillietina tetragona*; *Gallus gallus*; SDS-PAGE; proglottids; cestode.

1. INTRODUCTION

Cestodes are endoparasitic helminths and they represent a unique development system. Proglottids formation occurs continuously at neck region, youngest proglottids are present at the anterior region

and the oldest proglottids are at the end of tapeworm, they exhibit pseudo-metamerism. A continuous gradient of metabolites exists along the entire length of the body; representing developmental transition, differentiation and eventual degeneration of reproductive organs. During this transition,

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morphological changes are accompanied by physiological and biochemical changes [1]. The present study was undertaken to know the regionally specific protein expression in different proglottids during the transition.

Molecular approaches are the most effective and accurate means for the detection of many organisms and even for screening of genetic variation among populations [2]. Survey of literature on various aspects of biochemistry of cestodes reveals that they exhibit variation in different regions of the body, the degree of maturation in different regions results in the variation. A variation in protein profiles of *Cysticercus tenuicollis* has reported [3].

Until recently taxonomy of cestodes has been exclusively on conventional morphological grounds. But electrophoretic analysis of tissue protein has been used as a taxonomic tool in many organisms. It has been used to distinguish different regions of organisms to understand the structure and functional significance.

Protein banding patterns from *Hymenolepis diminuta* was studied [4] and proteins of *Moneizia expansa* has analysed by employing electrophoresis [5]. Electrophoresis was employed in various manners like studying the banding pattern of sheep cestodes' total protein and the variation too.

It was also employed for identification of worms irrespective of their different developmental stages on the basis of protein profile patterns in *Taenidae* [6] and differentiation of bile duct cestodes' of marsupials' was successfully proven electrophoretically [7]. *Taenia hydatigena* has characterized by electrophoresis methods [8]. Protein expression patterns of helminths and different protein antigens were isolated by SDS-PAGE (Sodium Dodecyl Sulphate-Poly Acrylamide Gel Electrophoresis) [9]. Protein profiles and sero-diagnosis of *Taenia solium* bladderworm were reported [10]. Protein expression patterns of helminths of different age were relatively studied with respect to age [11]. No reports are available on the differentiation of proglottids of *Raillietina tetragona* by employing SDS-PAGE, therefore the present work has been undertaken to study the proglottids differentiation and to know the differential expression of proteins with respect to their regional specification.

2. MATERIAL AND METHODS

The investigation was carried out in between 2011 – January 2013. The intestines of freshly slaughtered chicken were collected from Warangal region of

Telangana, India and screened for *Raillietina tetragona* infection. Some of the worms were processed for taxonomic identification by following standard methods. Identification was carried out with the help of Systema Helminthum volume-II [12]; Helminths, arthropods and protozoa of domesticated animals [13].

Entire worms of same length were selected and immature, mature and gravid regions were located and separated for further analysis. Immature proglottids containing scolex, neck and anterior region, mature region having functional and reproductively active segments and gravid region laden with only eggs were separated.

Separation of proteins was performed by SDS PAGE [14], the worm were washed with phosphate buffer of pH 7.4, weighed and homogenized by homogenizer in tris -HCl buffer pH (7.4, 0.01M) containing 0.1% homogenate was centrifuged for 20 minutes at 2000 rpm. The supernatant was drawn and an equal volume of solution of sample loading buffer (pH 6.8) containing SDS, β ME (β -Mercapto Ethanol) and Bromo phenol blue was added [15].

3. RESULTS

A complex set of almost 14 bands of peptides ranging in the size from approximately 10,250-95,720 KDa(Kilo Dalton) was detected in different proglottids under reducing SDS-PAGE condition (Fig. 1). In the present study, an interesting observation was the presence of some additional polypeptides in a regionally specific manner in the range of 95,720; 55,080; 42,660; 25,700; 16,980 and 10,250 Da MW and the protein pattern of three different regions was presented as Fig. 1.

4. DISCUSSION

A new band in the range of 95,720 Da was observed in mature region, which could be attributed to amplified metabolic activity in mature region so as to produce more amounts of proteins required for production of eggs and reproductive activities. Bands in the range of 55,080; 42,660 Da were induced both in mature and gravid regions, which may be involved in reproductive activity.

In the present study two bands with 25,700 and 16,980 MW in immature segment were observed, and this could be attributed to amplified metabolic activity of immature region so as to produce more amount of proteins required for metabolic activity for differentiation in a regionally specific manner as they are involved in different functions at different rates

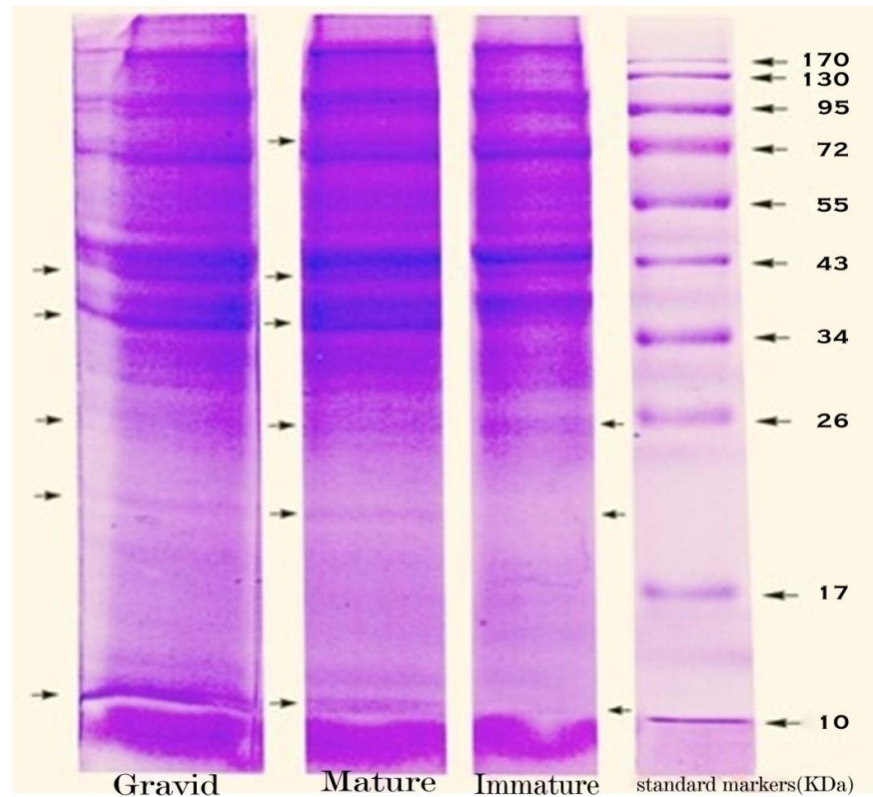


Fig. 1. Zymogram showing the protein bands (from left to right; gravid, mature, immature proglottids and standard molecular markers on extreme right)

SDS-PAGE was used to characterise and compare different proglottids. The protein sample loaded was 50 µg in each well, the molecular marker ladder of 170,130,95,72,55,43,34,26,17,10 KDa MW (molecular weight) were used.

in different stages of development. It is in contrast with some reports [16]. Protein band in the range of 25,700 and 10,250 Da was observed in mature and gravid regions, while degenerated to some extent in immature region indicating the transition and it could be attributed to its negative correlation with reproductive functions. The banding patterns of all the regions were similar at some regions. The observation of high degree of conservation of peptide components of defined molecular weight suggests that these proteins may be involved in metabolism and structural complexity. Meanwhile, the disappearance of some bands in gravid region could be due to degenerative activities in gravid region. This may be correlated with the degeneration of reproductive organs in gravid region and absence of scolices in mature and gravid segments may also cause degeneration.

5. CONCLUSION

Proteins show relatively large variation among proglottids of worm in terms of quantity. The newly formed protein bands in mature region suggests

metabolic transition from immature to mature region which could be correlated with its reproductive activities *i.e* synthesis of proteins required for egg production and formation of organs. The disappeared bands in gravid region indicate the degenerative functions of that region as it is solely concerned with storage of egg. The newly formed proteins may have potential role in survival, adaptation and host parasite interactions. Detailed further analysis of the newly formed and disappeared bands is required for understanding their role in differentiation of proglottids may help in designing drugs to inhibit either differentiation at mature or at gravid region.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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