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DETERMINATION OF THE COMPATIBILITY BETWEEN *BIOMPHALARIA ALEXANDRINA* AND *ECHINOSTOMA LIEI* USING SDS-PAGE ANALYSIS OF TISSUE SOLUBLE PROTEINS

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ABSTRACT

SDS-PAGE analysis of tissue soluble proteins of *Biomphalaria alexandrina* and rediae, cercariae, metacercariae and adult worms of *Echinostoma liei* was done for determination of the degree of compatibility between the snail and each of intramolluscan larval stages and adult worms of the parasite. The highest degree of similarity was observed between *B. alexandrina* and metacercariae of *E. liei.* However, the lowest degree of similarity was noted between *B. alexandrina* and adult worms of *E. liei.* On the other hand, the similarity coefficient between *B. alexandrina* and *E. liei* rediae was higher than that between the snail and *E. liei* cercariae.

INTRODUCTION

The echinostome worm, *Echinostoma liei* could be readily obtained in the laboratory from chicks, ducklings, hamsters and rats. Its natural final host in or near irrigation ditches of Nile delta involves the roof rat, Egyptian giant shrew and some aquatic birds. *Biomphalaria alexandrina* could be either naturally or experimentally infected with *E. liei* and so harboured the developmental stages of the parasite in the heart aorta and the digestive gland, while the metacercariae become encysted in the pericardium and kidney of the snail (Jeyarasasingam *et al.*, 1972).

The host immune response is an important line of defense against parasites. Tactics to evade this response are therefore expected in host-parasite relationships. Many parasites seem to

a degree of molecular camouflage against the host's immune system. Damian's theory, adopting a mechanism by which parasites could avoid the host immune response by mimicing host molecules, has greatly influenced both the theoretical and practical approaches to immuno-parasitology (Damian, 1987).

Larval trematodes are known to share molluscan host antigens in several parasite-host systems. Therefore it has been suggested that such a sharing of specific antigens may be responsible, at least in part, for regulating immune compatibility between parasites and their snail hosts (Wright, 1971; Basch, 1976; Lackie, 1980; Rasmussen *et al*., 1985 and Heyneman *et al*., 1985)

The present work aims to study the degree of similarity (compatibility) between *Biomphalaria alexandrina* and the adult worms as well as the intramolluscan larval stages of *Echinostoma liei* using electrophoretic analysis of tissue soluble proteins.

MATERIAL AND METHODS

Laboratory bred *B. alexandrina* snails (5-6mm shell diameter) were obtained from Schistosome Biological Supply Program (SBSP), Theodor Bilharz Research Institute, Imbaba, Giza, Egypt. The life cycle of *E. liei* was maintained in the laboratory for getting the adult worms and intramolluscan larval stages by applying techniques of Jeyarasasingam *et al*.(1972) and El-Dafrawy (2001). Redae, cercariae and metacercariae were obtained by dissection of infected *B. alexandrina,* using fine needle and forceps under a binocular microscope. *E. liei* adult worms were obtained from dissected infected albino rats *Rattus norvegicus,* ten days after ingestion of metacercariae.

Preparation of tissue extract and electrophoretic analysis was done according to the procedure of Maeda *et al*. (1984). Determination of the molecular weights of separated bands and calculation of the similarity coefficients were carried out using the methods previously employed by Mostafa *et al*. (2000).

RESULTS

The electrophoretic pattern of tissue soluble proteins extracted from *B. alexandrina* and the rediae, cercariae, metacercariae as well as adult worms of *E. liei* were shown in Figure (1) and the molecular values of separated bands were listed in Table (1).

The total number of separated electrophoretic bands was 19, 20, 19, 21 and17 bands for *B. alexandrina*, rediae, cercariae, metacercariae and the adult worms of *E. liei* respectively. The lowest molecular weight of bands was 29.44 KDa for all examined samples except for *E. liei* adult worm was the lowest molecular weight was 38.14 KDa. The highest molecular weights were 217.29, 216.00, 215.36, 217.29 and 216.00 KDa for *B. alexandrina*, rediae, cercariae, metacercariae and adult worms respectively. The number of shared bands between *B. alexandrina* and rediae, cercariae, metacercariae and adult worms of *E. liei* was 9, 7, 11 and 3 respectively. On the other hand, the similarity coefficients were 0.47, 0.37, 0.55 and 0.16 as calculated between *B. alexandrina* and each of the rediae, cercariae, metacercaria and adult worms of *E. liei* respectively.

DISCUSSION

The present observations were implicated in the concept of host-parasite antigen mimicry which was studied by a number of authors using different techniques. Historically, Fairley (1919 and 1920) was the first to observe the presence of shared antigens between schistosomes and their snail hosts by utilizing an extract from *"Planorbis"* (= *Biomphalaria*) *boissyi* infected with *S. mansoni* to diagnose human and experimental monkey infections. He noted that two monkeys immunized with cercarial extract, then infected with *S. mansoni* cercariae, reacted with uninfected snail "liver" antigen and commented that for some reason the cercarial challenge immunized the monkey against the snail antigens. Latter, the demonstration of five precipitin bands common between *B. glabrata* and *S. mansoni* was the study that clearly demonstrated that these were specific components shared between the parasite and its intermediate host Capron *et al.* (1965).

Further evidences of shared antigens between parasites and their molluscan hosts were provided by subsequent papers.

Using immunoflurescence and immunoelectron microscopical methods, Yoshino and Bayne (1983) have demonstrated that antibodies to susceptible and resistant *B. glabrata* haemolymph cross react with *S. mansoni* miracidial epidermal and ciliary membranes as well as the surface membranes of intercellular ridges. Primary sporocysts, both transformed in vitro and maintained in culture for

various time intervals in the absence of snail-derived factors, retain haemolymph-like antigens on their surface tegument although at reduced levels in comparison to miracidial stages. Since miracidia and sporocysts were derived in media devoid of snail host materials, shared antigens on larval surfaces are believed to be of parasite origin and constitute true molecular mimicry as defined by Damain's theory, 1987. Rasmussen *et al.*(1985) using SDS-PAGE and Western blot analysis, demonstrated the presence of shared antigens between *B. glabrata* and both *S. mansoni* and *Fasciola hepatica.* Immunoelectron microscopical studies done by Dissous *et al.* (1990) indicated that antibodies to *S. mansoni* (Sm 39) specifically bound to muscular structures of *S. mansoni* worms and its intermediate host *B. glabrata* snails. Molecular cloning and sequencing indicated that the cross-reactive proteins of *B. glabrata* (Bg 39) corresponded to a uscular isoform of tropomyosin. The mollusc sequence showed a 51-65% homology with seven different musculer tropomyosins from vertebrate and invertebrate species. The highest score of homology was observed with *S. mansoni* tropomyosin, suggesting that cross-reactive determinants could be specific for the trematode and its intermediate host. Moreover, in miracidia, Sm39 epitopes were also shown to be contained in the vesicles present in the epidermal ridges and cellular bodies. Such vesicles are involved in the formation of a protective tegument around sporocysts, suggesting a possible role of cross-reactive tropomyosins in miracidia and/or sporocyst-snail interaction.

Mukaratirwa *et al.* (1996) studied the genetic structure (as determined by allozyme genetics) of eight populations of *Bulinus globosus* from 2 areas of different endemic city of *S. haematobium.* They made a correlation between the level of genetic variability of different populations of *B. globosus* and *S. haematobium* infection rate.

Knight *et al.* (2000) determined the molecular basis of *B. glabrata-S. mansoni* relationship using random amplification of polymorphic DNA-PCR-based technology, with restriction fragment length polymorphism analysis and the generation of expressed sequence tags from the snail.

In the present investigation, SDS-PAGE analysis of tissue soluble proteins of *B. alexandrina* snails, rediae, cercariae, metacercariae and adult worms of *E. liei* was done to determine the degree of similarity between the intermediate host and the parasite larval stages and adult worm. The present work demonstrated that the

similarity coefficient between *B. alexandrina* and *E. liei* rediae was higher than that between *B. alexandrina* and *E. liei* cercariae. Rediae were observed in the ovotestis of the snails by day 15-21 post-exposure to miracidia (Jeyarasasingam *et al.*, 1972). Rediae feed on the tissue of their intermediate host using their muscular pharynx to pump food into the gut and also obtain nourishment by transtegumentary absorption. So both their gastrodermis and tegument are highly modified for nutrient uptake. On the other hand, cercariae were considered as juvenile, non-feeding stage with non-functional gut (Fried and Haseeb, 1991). Therefore, in addition to the expected mimicking host molecules on the surface of rediae, they were contained host tissues within their gut leading to the high degree of similarity between *B. alexandrina* and *E. liei* rediae. On the contrary, the degree of similarity between *B. alexandrina* and cercariae of *E. liei* was lower since, the cercariae contained the mimicked molecules only.

The highest degree of similarity was recorded between *B. alexandrina* and *E. liei* metacercariae (0.55). *E. liei* cercariae encyst in *B. alexandrina* which acts as a second intermediate host (Jeyarasasingam *et al.*, 1972). Therefore, such highest degree of similarity could be due to the contribution of *B. alexandrina* in the cyst wall formation of *E. liei* metacercariae. This conclusion was correlated with Fried and Haseeb (1991) who reported that, the free-encysting metacercariae produce numerous layered cysts of parasitic origin and do so more rapidly than their counterparts encysting in the host tissues. In the latter ones, the host may contribute secretion to cyst wall formation. Gulka and Fried (1979) found that the cercariae of *E. revolutum*, which encysted in the tissues of an intermediate host, may take up to a day for the cyst to harden and become infective to the definitive host. Moreover, the intermediate host contributes substances (e.g collagen) to the cyst wall of the metacercaria.

The lowest similarity coefficient (0.16) -in the present work- was obseved between *B. alexandrina* and *E. liei* adult worm. The rediae, cercariae and metacercariae of *E. liei* were shared identical environment within *B. alexandrina*, so their similarity coefficient with the snail was relatively high. However, *E. liei* adult worms were found in completely different environment (the definitive host) and as a result their similarity coefficient with *B. alexandrina* was much lower.

The present results and conclusions were more or less correlated with that of Martin *et al.* (2001) on their work on *Varroa jacobsoni* the ectoparasitic mite of honey-bee *Apis mellifera* to study the chemical minicry. They studied the cuticular hydrocarbon patterns of both parasite and host at different stages of bee development. Cuticular components were identified by gas chromatography/mass spectrometry. The proportion of each component was calculated at three stages of bee development (larvae, pupa, and emerging bee). The degree of chemical mimicry between the parasite and host was evaluated by multivariate analyses using the resulting proportions for each category of individuals. They reported four main findings. The first was that the proportions of some components are different at larval, pupal and imago stage of bee development. Second, *V. jacobsoni* profiles vary depending on the developmental stage of the host. Third, the cuticular profile of adult mites is more similar to that of the stage of the host than that of later and/or earlier stages except for parasites collected from emerging adult bees. Fourth, the degree of mimicry by mites is greater during larval and pupal stages that during the emerging adult bee stages.

In addition, the antigenic mimicry as a mechanism for escaping the host immune system has been studied in other host-parasite systems.

Murine adenovirus, Semliki forced virus, lactate dehydrogenase-elevating virus, herpes simplex virus type-1, hepatitis B virus, encephalomyocarditis virus, Theiler's murine encephalomyelitis viruses, coxsackie virus and cytomegalovirus have been found to mimic physiologically important host proteins. Molecular mimicry of viral antigens with self-determinant has been proposed as one of the pathogenic mechanisms in autoimmune disease (Lawson, 2000).

Trypanosoma cruzi, the agent of Chagas' disease, expresses trans-sialidase, a unique enzyme activity that enable the parasite to invade host cell by transferring sialyl residues from host glyconjugates to the parasite's surface acceptor molecules. Epitope mapping of trans-sialidase from *T. cruzi* revealed the presence of several cross-reactive determinants (Pitcovsky *et al.*, 2001).

The present work is so far the first dealing with the determination of degree of similarity (compatibility) between *B. alexandrina* and *E. liei* larval stages and adult worms using SDS-PAGE analysis of tissue soluble protein. Further studies will be done using the immunological and other molecular techniques.

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Table (1) Molecular weights of tissue soluble protein fractions separated by SDS-PAGE for clean, non infected *Biomphalaria alexandrina* (A), *Echinostoma liei* redia (B), cercariae (C), metacercariae (D), and adult worms (E).

No	(A) *Biomphalaria alexandrina*	(B) *Echinostoma liei* redia	(C) *Echinostoma liei* Cercariae	(D) *Echinostoma liei* Metacercariae	(E) *Echinostoma liei* Adult worms
1	217.29	216.00	215.36	217.29	216.00
2	213.44	213.44	213.44	213.44	211.19
3	208.41	208.41	206.55	205.33	203.53
4	205.33	203.50	200.49	203.50	196.43
5	199.30	199.30	191.76	199.30	190.98
6	194.15	190.82	183.88	194.15	183.88
7	183.88	183.88	173.30	190.82	175.52
8	173.30	175.02	167.41	182.52	169.92
9	163.74	169.91	147.93	176.32	159.32
10	146.81	145.71	128.09	167.83	145.01
11	132.04	127.13	110.92	149.06	132.04
12	109.25	109.25	96.86	125.55	120.13
13	91.52	97.28	84.25	109.25	144.01
14	78.23	87.62	81.01	96.44	89.45
15	71.38	78.23	71.38	90.73	63.29
16	66.28	68.94	66.28	81.71	47.89
17	51.72	61.16	50.17	71.38	38.14
18	38.14	51.72	38.14	66.28	
19	29.44	38.14	29.44	51.72	
20		29.44		38.14	
21				29.44	

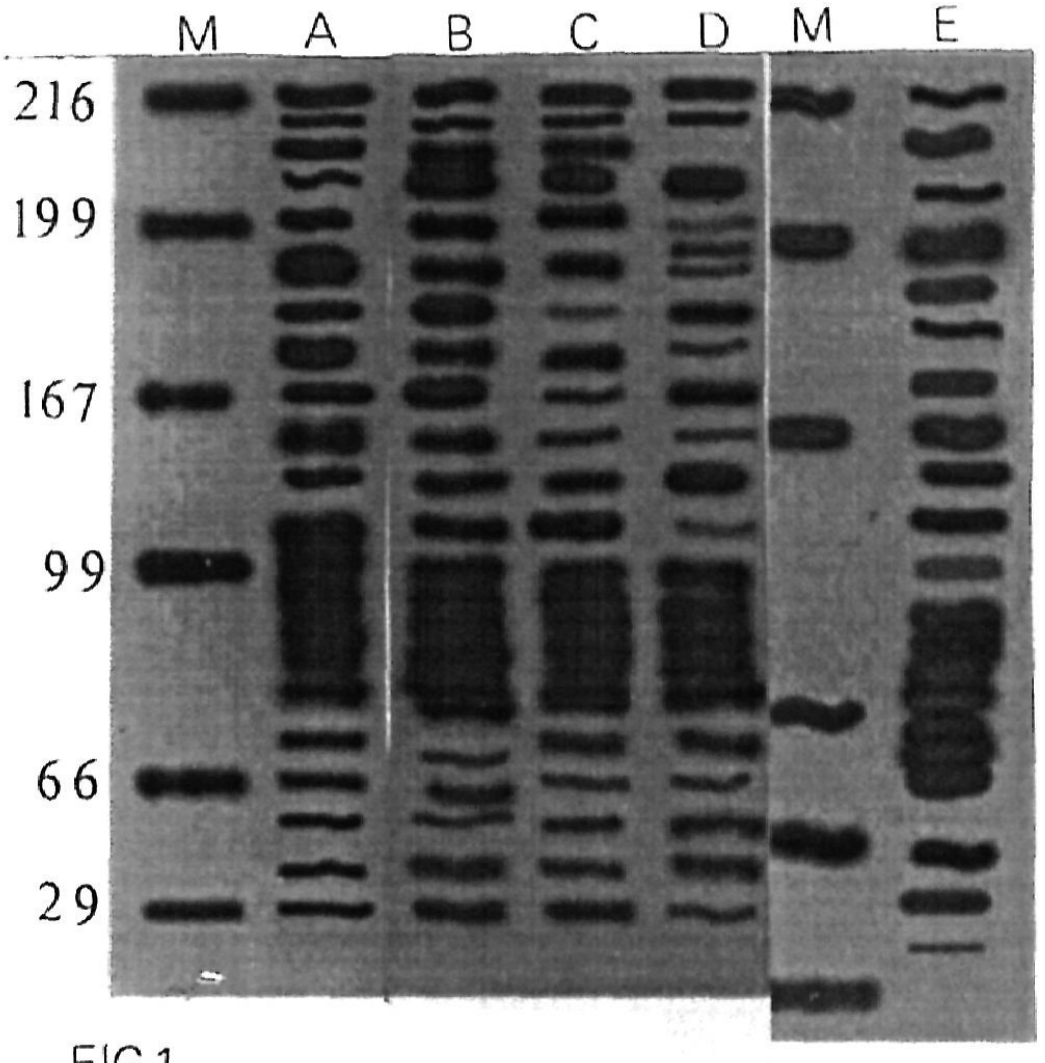

FIG.1

Fig. (1): SDS-PAGE pattern of tissue soluble proteins of *Biomphalaria alexandrina* (A) and rediae (B), cercariae (C), metacercariae (D) and adult worms (E) of *E. liei*. Lane (M) is the molecular weight marker.