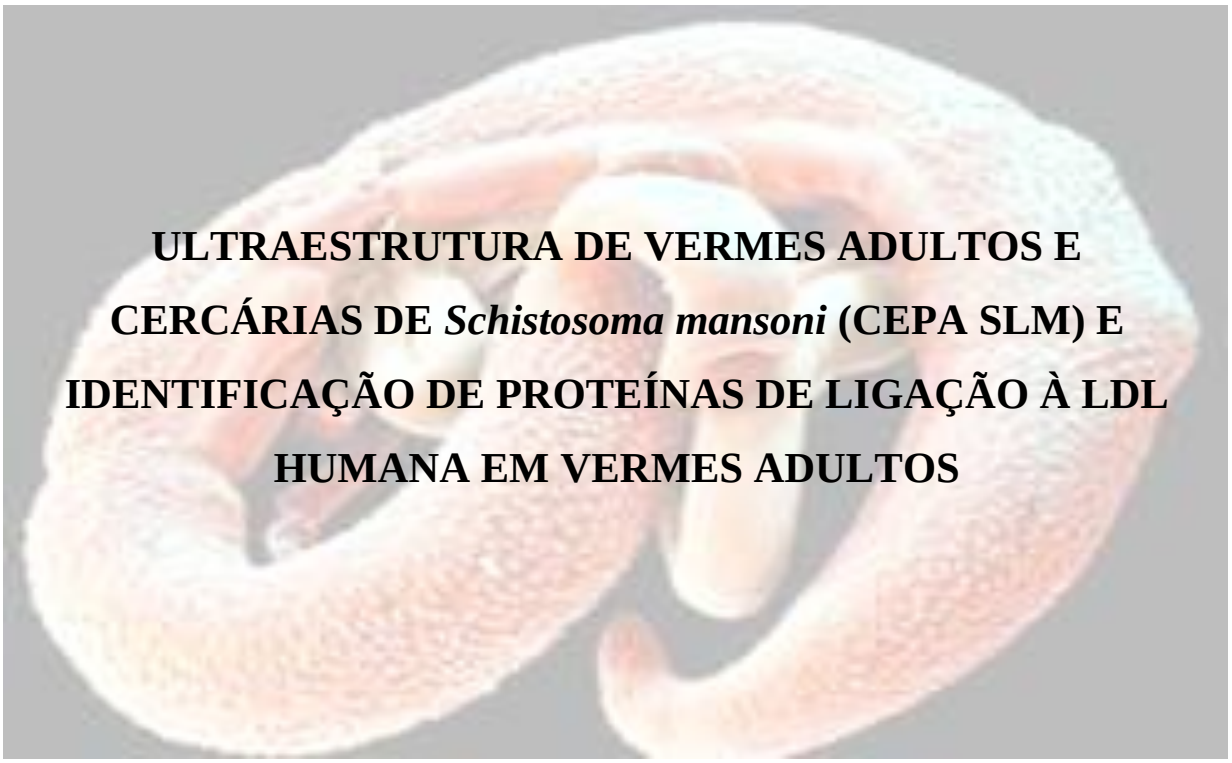
Universidade Federal de Pernambuco

Laboratório de Imunopatologia Keizo Asami

Programa de Pós-graduação em Biologia Aplicada à Saúde



ULTRAESTRUTURA DE VERMES ADULTOS E CERCÁRIAS DE *Schistosoma mansoni* (CEPA SLM) E IDENTIFICAÇÃO DE PROTEÍNAS DE LIGAÇÃO À LDL HUMANA EM VERMES ADULTOS

Adriana da Silva Andrade Pereira

Recife - PE

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Tese apresentada ao Programa de Pós-graduação em Biologia Aplicada à Saúde do Laboratório de Imunopatologia Keizo Asami – LIKA/UFPE, como requisito para a obtenção do título de Doutor em Biologia Aplicada à Saúde.

Orientador: Prof. Dr. José Luiz de Lima Filho

Co-orientadora: Prof. Dra. Maria Elizabeth Cavalcante Chaves

Recife - PE

2014

Catalogação na fonte
Elaine Barroso
CRB 1728

Pereira, Adriana da Silva Andrade
Ultraestrutura de vermes adultos e cercarias de *Schistosoma mansoni* (CEPA SLM) e identificação de proteínas de ligação à LDL humana em vermes adultos/ Recife: O Autor, 2014.

112 folhas : il., fig., tab.
Orientador: José Luiz de Lima Filho
Coorientadora: Maria Elizabeth Cavalcante Chaves
Tese (doutorado) – Universidade Federal de Pernambuco, Centro de Ciências Biológicas, Biologia Aplicada à Saúde, 2014.
Inclui bibliografia e anexo

1. **Esquistossomose 2. *Schistosoma mansoni* I. Lima Filho, José Luiz de (orientador) II. Chaves, Maria Elizabeth Cavalcante (coorientadora) III. Título**

614.553 CDD (22.ed.) UFPE/CCB- 2014- 187

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17/02/2014

DEDICATÓRIA

Com amor à minhas avós Rita e Ardale (*in memoriam*).

AGRADECIMENTOS

Em primeiro lugar, agradeço a Deus por tudo que tem realizado em minha vida, pela oportunidade e alegria de chegar até este momento. Por toda a força, conhecimento e saúde para a realização deste trabalho, assim como pelas pessoas maravilhosas que colocou no meu caminho.

Aos meus pais, *Marise Andrade* e *Aderaldo Andrade*, pelo zelo, amor, todo sacrifício que fizeram para que eu chegasse até onde cheguei e apoio incondicional ao longo dessa caminhada.

À minha querida família: meu irmão *Kleyton*, minha sobrinha amada *Ana Lívia* e minha cunhada *Moane*, pela confiança e por sempre estarem presentes na minha vida. À minhas tias *Mirian (in memoriam)* e Conceição *(in memoriam)*, meu avô Artur *(in memoriam)* por todo amor e ensinamentos valiosos.

Em especial agradeço às minhas queridas avós Ardale *(in memoriam)* e Rita por todo exemplo de garra e superação, por serem pilares na minha vida, onde sempre encontrei um porto seguro, muito amor, apoio, conselhos preciosos e por me mostrarem o que realmente é essencial na vida.

À Professora *Elizabeth Chaves*, que foi minha orientadora, e a quem devo minha iniciação à ciência, por toda sua paciência, dedicação, amizade, confiança depositada para realização deste trabalho, tempo dispensado em ensinamentos preciosos, não só científicos, mas também humanos. E principalmente por estar sempre presente, meu profundo e sincero agradecimento.

Ao meu orientador *Prof. Dr. José Luiz de Lima Filho*, por nunca duvidar da capacidade de seus orientandos.

À Pós-graduação em Biologia Aplicada à Saúde: *Prof. Luiz Carvalho* e *Eliete Rodrigues* pelo total suporte e apoio. Aos funcionários do LIKA: *Ilma Santos, Verinha, Seu Otaviano* e *Edson.*

Aos amigos do Laboratório de Microscopia Eletrônica do LIKA: *Rafael Padilha* e *Marília Gabriela* que deram grande contribuição para a realização deste trabalho, sendo sempre atenciosos e prestativos.

À Unidade de Espectrometria de Massas e Proteômica da Universidade Federal do Rio de Janeiro representada pela *Prof. Russolina Zingali* e *Ana Lúcia* pelo acolhimento, apoio e ensinamentos.

Amigo é a família que te acolhe e em especial agradeço à: *Gabriela Ayres, Luiza Lima, Ana Luiza, Ana Carolina, Amanda Rafaela, Ivana Porto, Anabelle Bezerra, Natália Lima, Tamara De'Carli, Marcela Outtes* e *Telma Valkíria*. Mesmo muitas vezes tão distantes, estamos sempre tão perto. Obrigada por fazerem parte da minha vida e lembrando que nenhum caminho é longo demais quando um amigo nos acompanha.

Aos amigos queridos do LIKA: *Roberto Afonso, Isabella Macário, Milena Sales, Fábio Fidélis, Taciana Salviano, Mariana Cabrera, Lúcia Patrícia, Sinara Mônica, Monique Ferraz* e *Mariana Arruda*.

Ao CNPq e a CAPES pelo suporte financeiro do projeto.

Enfim, meu muito obrigado a todos que de alguma forma contribuíram direta ou indiretamente para a conclusão deste trabalho.

RESUMO

Schistosoma mansoni, é um dos mais importantes parasitas que infectam seres humanos. No nordeste do Brasil, a esquistossomose é historicamente endêmica e considerada como um problema de saúde pública. No estado de Pernambuco, particularmente no litoral e zona da Mata, predomina a cepa São Lourenço da Mata (SLM). Estudos ultraestruturais desta cepa até então ainda não foram realizados, embora análises morfométricas e morfológicas de outras cepas existentes no Brasil já tenham sido publicadas na literatura. Os vermes são bem adaptados ao hospedeiro, tendo a sua longevidade vista como uma conseqüência do eficaz escape do sistema imune. Na circulação sanguínea os vermes devem obter lipídios a partir do hospedeiro, já que eles não sintetizam tais compostos, sendo as lipoproteínas plasmáticas a possível fonte de tais lipídios, além de poderem atuar mascarando o reconhecimento do verme pelo sistema imune. Neste trabalho, microscopia eletrônica de varredura (SEM) foi utilizada para a análise morfológica e morfométrica de cercárias e vermes adultos de *S. mansoni* cepa SLM, bem como para estudar a interação da lipoproteína de baixa densidade (LDL) com o tegumento do parasito. Também foram usadas diferentes técnicas para a localização e identificação das proteínas envolvidas no processo de absorção e/ou transporte de lipoproteínas no verme adulto de *S. mansoni*. As cercárias foram obtidas de caramujos *Biomphalaria glabrata* e os vermes adultos de camundongos machos *Mus musculus* e *Swiss*, ambos infectados pela cepa SLM de *S. mansoni*. Os resultados da SEM mostraram que os corpos da cercárias são cobertos por espinhos, com uma ventosa ventral, uma ventosa oral com receptores sensoriais, e um par de glândulas de penetração na cabeça. Seu comprimento total variou de 174 a 290µm. O comprimento dos vermes adultos machos foi de 4mm e das fêmeas 5mm. O comprimento da região anterior do macho foi de 470µm e 271µm para o sexo feminino. Todos os parâmetros foram realizados em dez amostras. Os valores encontrados na morfometria da cepa SLM foram menores que em outras cepas de *S. mansoni* já descritas. Através da microscopia eletrônica de transmissão (TEM), os experimentos de imunomarcação mostraram partículas de ouro localizadas dentro do tegumento, na região muscular e espículas dos machos e em torno das células vitelínicas das fêmeas. A utilização de técnicas tais como imunoblotting e "liquid chromatography–mass spectrometry" (LC-MS) proporcionaram a identificação no *S. mansoni* de duas formas diferentes da chaperona Hsp 70, proteínas envolvidas na interação com a apo-B e o seu transporte para o retículo endoplasmático, sendo responsável também pela ruptura das interações clatrina-clatrina na endocitose humana. Com a descoberta destas proteínas, novos estudos serão necessários para esclarecer o papel funcional das mesmas no *S. mansoni*.

Palavras chaves: Hsp 70, Interação de LDL, Lipoproteínas, Estudo Morfométrico, Schistosoma, *Schistosoma manosoni*.

ABSTRACT

Schistosoma mansoni is one of the most important parasites that infect humans. In the northeastern of Brazil, schistosomiasis is endemic and considered a public health problem. The worms are well adapted to the host, and their longevity seems to be a consequence of effective escape of the immune system. The lipoproteins of the human plasma, act as a lipid source for Schistosoma and help to hide them from the attack by antibodies present in the circulation. The São Lourenço da Mata (SLM-PE, Brazil) strain of *S. mansoni* has been used in several publications, but morphological and morphometric studies about this strain were never done. The cercariae were obtained from *Biomphalaria glabrata* snails and the adult male worms from *Swiss* mice, both infected by *S. mansoni* SLM strain. In this work, scanning electron microscopy (SEM) was used in morphological and morphometric analysis of cercariae and adult worms of *S. mansoni* SLM strain, as well as to study the interaction of low density lipoprotein (LDL) with the parasite tegument. It was also used different techniques to location and identification of proteins involved in the uptake of lipids and/or transport of LDL in *S. mansoni*. The SEM results from cercariae showed that the bodies are covered by spines, with a ventral sucker, an oral sucker, sensory receivers, and a pair of penetration glands in the head. The area of tail and body and the distance between suckers were 3,011.77μm, 1,530.32μm, and 42.9μm, respectively. Adult worms of *S. mansoni* were divided into three main regions: the anterior, medial, and posterior, besides the gynecophoral canal in males. The measure of adult worms of *S. mansoni* was 4mm males and 5mm females. The anterior region length of the male was 470μm and of the female 271μm. All the parameters were assayed in ten samples. The morphometric values found in the SLM strain were smaller than other *S. mansoni* strains already described. Through the transmission electron microscopy, the immunolabelling experiments showed gold particles localized within the tegument, muscle region and spines in male worms and around vitellin cells in the female. The use of techniques such as immunoblotting and LC-MS provided the identification of two different forms of the chaperone Hsp 70 in the *S. mansoni*, proteins involved in the interaction with apo-B and its transport to the endoplasmic reticulum, being responsible for disrupts clathrin-clathrin interactions. With the discovery of those proteins, studies are needed to clarify the lipoprotein-protein interactions, as well as the functional role of the same. Studies concerning to *S. mansoni* are extremely important for a better understanding of the parasite biology, thus improving control strategies to the schistosomiasis.

Keywords: Hsp70, Interaction of LDL, Lipoproteins, Morphometric study, Schistosoma, *Schistosoma mansoni*.

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1. INTRODUÇÃO

A esquistossomose é uma parasitose endêmica, uma das mais importantes doenças tropicais negligenciadas, que afeta cerca de 230 milhões de pessoas em 77 países (Engels et al., 2002; Van der Werf et al., 2003; Gryseels et al., 2006; WHO, 2013). No Brasil, esta parasitose continua sendo uma doença importante no contexto da saúde pública, encontrando-se em franca expansão por falta de uma política de saneamento que melhore as condições de vida das populações principalmente, as de baixa renda (Vitorino et al., 2012; Brasil, 2005). No nordeste a esquistossomose tem sido amplamente distribuída e Pernambuco é um dos estados com a maior prevalência da doença, estando presente em 103 dos 186 municípios, principalmente na zona da mata, litoral e em cidades das margens do rio Capibaribe (Barbosa et al., 1998; Katz & Peixoto, 2000; Barbosa et al., 2001; FUNASA, 2002; Brasil, 2011).

A esquistossomose é causada por vermes trematódeos do gênero Schistosoma. Uma das três principais espécies que infectam os seres humanos é o *Schistosoma mansoni* (Gryseels et al., 2006). A doença causa um severo grau de morbidade e mudanças patológicas, afeta o sistema vascular porta-mesentérico para onde os vermes migram e iniciam a oviposição. Parte dos ovos depositados pela fêmea, dentro do mesentério ficam presos no fígado, que acaba por comprometer a função hepática e assim resultando nos sintomas característicos da doença como: emagrecimento, endurecimento e o aumento de volume do fígado, febres, hemorragias, entre outros (Loeffler & Bennett, 1996). O Schistosoma persiste no sangue durante décadas, eles possuem um método eficaz de evasão imune que é dependente das propriedades do tegumento parasito (Abath & Werkhauser, 1996; Schramm & Haas, 2010).

O tegumento do Schistosoma é uma camada interativa e dinâmica que está envolvida na complexa relação hospedeiro-parasita e em processos de nutrição, evasão imune, excreção, osmorregulação, recepção sensorial e transdução de sinal (Jones et al., 2004; Van Hellemond et al., 2006; Pérez-Sánchez et al., 2008; Mulvenna et al., 2010; Xavier et al., 2010). A sobrevivência do parasito depende da formação contínua de novas membranas e estas requerem lipídios do hospedeiro para sua construção (van Hellemond et al., 2006). O Schistosoma é incapaz de sintetizar colesterol *de novo* e

ácidos graxos (Meyer et al., 1970). Vários estudos têm demonstrado a expressão de proteínas em protozoários, helmintos, esquistossômulos e vermes adultos de Schistosoma, que são capazes de ligar lipoproteína de baixa densidade (LDL), lipoproteína de muito baixa densidade (VLDL) e apoproteína B (apo B) (Rumjanek et al., 1983, 1985; Chiang & Cauldfield, 1989; Rogers et al., 1989; Tempone et al., 1997). Assim, não é surpreendente que existam receptores endógenos, expressos na superfície das membranas que possam desempenhar papéis importantes, tais como absorção de lipídios de lipoproteínas do plasma do hospedeiro (Acton et al., 1994).

A endocitose da LDL mediada por clatrina em seres humanos é um processo complexo que envolve proteínas reguladoras e acessórias, entre as quais está a proteína de choque térmico 70 (Hsp 70). A literatura indica que muitas proteínas podem interagir com Hsp 70 em humanos, ela é responsável por quebrar as interações clatrina-clatrina, levando à liberação do conteúdo da vesícula (Beckmann et al., 1990; Hendrick & Hartl, 1993). A Hsp 70 é uma proteína citoplasmática que tem a capacidade de se ligar a polipeptídeos de domínios hidrófobicos, além de participar do transporte de proteínas para as organelas celulares (Walter & Lingappa, 1986; Frydman et al., 1994; Zhou et al., 1995; Mayer & Bukau, 2005). A Hsp 70 encontra-se expressa em algumas das fases do ciclo de vida do *S. mansoni* (miracídio, esporocisto, esquistossômulo e verme adulto), sendo sugerida sua participação no processo de endocitose de LDL (Holtzman & Schechter, 1996; Kanehisa, 2000).

Muitos mecanismos têm sido propostos para explicar a sobrevivência do parasita no homem e em outros mamíferos. As inúmeras dúvidas sobre o comportamento do parasita no hospedeiro definitivo demonstram que muito tem que ser feito para entender o seu metabolismo. Por outro lado, a bioquímica tem avançado nas pesquisas em proteínas principalmente, com o advento da análise proteômica, possibilitando que proteínas sejam estudadas e suas sequências estabelecidas (Delcroix et al., 2007; Schmid-Hempel, 2009; Silva et al., 2012).

Outro ponto importante para um melhor conhecimento do parasita são os estudos morfológicos e morfométricos, que têm por objetivo o esclarecimento da taxonomia de várias espécies pertencentes a diferentes cepas de *S. mansoni* (Machado-Silva et al., 1994; Oliveira et al., 2003). Com a evolução tecnológica dos equipamentos de análise estrutural, um estudo detalhado das regiões dos parasitas pôde ser feita. Vários autores

utilizam a morfometria de vermes adultos e cercarias para caracterizar cepas de *S. mansoni*, pois os resultados podem apresentar diferenças dependendo da cepa ou espécie (Magalhães et al., 1973; Machado-Silva et al., 1995, 2000).

2. OBJETIVOS

Os objetivos deste trabalho serão descritos a seguir.

2.1 GERAL

Caracterizar morfométrica e morfologicamente cercárias e vermes adultos de *Schistosoma mansoni* cepa São Lourenço da Mata (SLM). Localizar os sítios de ligação de lipoproteínas de baixa densidade (LDL) humana sobre o tegumento do verme adulto macho de *S.mansoni* e identificar nesta cepa, proteínas que interajam com a LDL humana.

2.2 ESPECÍFICOS

- Caracterizar e descrever por microscopia eletrônica de varredura a morfologia e morfometria de cercárias e vermes adultos de *Schistosoma mansoni* da cepa São Lourenço da Mata (SLM);
- Localizar os sítios de ligação de lipoproteína de baixa densidade (LDL) no tegumento de vermes adultos de *S. mansoni* através da microscopia eletrônica de varredura;
- Cortes ultrafinos, para identificar por microscopia eletrônica de transmissão sítios de ligação no verme adulto de *S. mansoni* capazes de interagir com a LDL humana e/ou componentes do soro humano identificados por anticorpo anti-LDL;
- Identificar "spots" correspondentes a(s) proteína(s) envolvidas no processo de ligação com a LDL humana no verme adulto de *S. mansoni* usando técnicas de eletroforese bidimensional e "Western blotting";
- Coletar as proteínas de interesse e analisar os fragmentos trípticos por LC-MS;
- Identificar as proteínas através de bancos de dados disponíveis na Internet.

3. REVISÃO BIBLIOGRÁFICA

3.1 *Schistosoma mansoni*

O *Schistosoma mansoni* é um parasita trematódeo de longa duração do sistema hepático portal humano (Ximenes et al., 2000). O seu ciclo evolutivo se desenvolve em duas fases principais: no caramujo (hospedeiro invertebrado ou intermediário) e no homem e outros mamíferos (hospedeiros vertebrados ou definitivos). No Brasil, os caramujos que servem como hospedeiros intermediários são do gênero *Biomphalaria* e das espécies *B. glabrata, B. straminea e B. tenagophila.* Destas, apenas as duas primeiras são encontradas no Nordeste brasileiro (Brasil, 2007).

3.1.1 Ciclo Biológico

Em seu ciclo biológico (Figura 1), os ovos do Schistosoma ao alcançarem a água, eclodem na presença de luz e calor, e liberam os miracídios, larvas ciliadas, que nadam ativamente até encontrarem o hospedeiro intermediário. O desenvolvimento das larvas do parasita em esporocistos primários, esporocistos secundários e cercárias (larvas infectantes) dura de vinte a trinta dias (Rey, 2001; Neves, 2005). A penetração das cercárias na pele do hospedeiro vertebrado inicia a fase de desenvolvimento parasitário no hospedeiro definitivo e há, contudo uma considerável diferença na intensidade da infecção e morbidade (Clerinx & Van Gompel, 2011). Durante a penetração, as larvas infectantes se transformam em esquistossômulos e migram através dos vasos sanguíneos ou linfáticos para os pulmões e, finalmente para o sistema vascular intra-hepático. Esse processo dura em torno de duas semanas, enquanto a maturação dos parasitas até a forma adulta é alcançada 30 - 45 dias após a penetração (Rey, 2001).

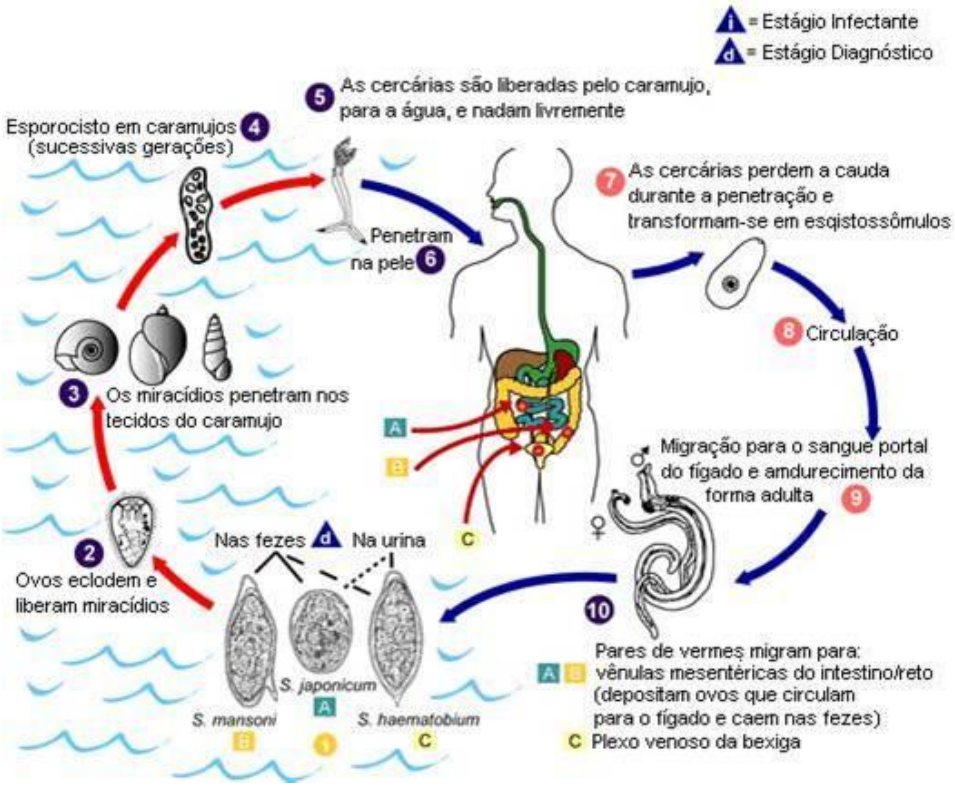


Figura 1: Ciclo de vida das três espécies de *Schistosoma*

Fonte: http://www.cve.saude.sp.gov.br/htm/hidrica/IF_ESQUI05.htm

(Endereço eletrônico acessado dia 24 de janeiro de 2014)

3.1.2 Morfologia das Cercárias

As cercárias (Figura 2) são a fase evolutiva infectante do *Schistosoma mansoni*. Apresentam-se como formas larvares multicelulares com caudas bífurcadas que possuem funções de locomoção, invasão do hospedeiro e de maturação em vermes sexualmente maduros. Seu comprimento é de aproximadamente 500µm, podendo variar devido sua habilidade de contrair-se ou alongar-se (Dorsey et al., 2002).

Devido ao vigoroso movimento durante a natação, contração e extensão da cauda, corpo e ventosas, as cercárias apresentam uma grande quantidade de células musculares. O corpo desta forma evolutiva apresenta-se dividido em: ventosa oral ou órgão anterior, segmento do corpo e cauda, terminando em uma bifurcação. Esta última

região da cercária será perdida rapidamente no seu processo de penetração no hospedeiro definitivo (Dorsey et al., 2002; Neves, 2005).

Figura 2: Micrografia Eletrônica de Varredura da Cercária de *Schistosoma mansoni*

Fonte: Pereira et al., 2013.

A estrutura básica do tegumento é semelhante na cercária, esquistossômulo e vermes adultos. A cercária jovem é coberta por um epitélio primitivo de 0,5µm de espessura. Este epitélio permanece até a fase embrionária e possui poucos núcleos e nenhuma junção celular é observada. Este tecido primitivo tem a função de proteger o tegumento verdadeiro até que o mesmo esteja formado (Hockley, 1972).

3.1.3 Morfologia dos vermes adultos

Os parasitos do gênero *Schistosoma*, pertencem ao Filo Platelminto e Classe Trematódea. Os vermes adultos exibem marcado dimorfismo sexual, com vermes mais

largos, os do sexo masculino apertando os mais finos, do sexo feminino, em um canal ou sulco ginecófaro (Han et al., 2009).

O verme adulto macho (Figura 3) mede cerca de 1cm, tem o tegumento recoberto de minúsculas projeções (tubérculos) (Braschi & Wilson, 2006). Apresenta o corpo dividido em três porções: anterior, na qual encontramos a ventosa oral e a ventosa ventral (acetábulo), a mediana e a posterior (que se inicia logo após a ventosa ventral), onde aparece o canal ginecófaro. Este nada mais é, do que dobras das laterais do corpo no sentido longitudinal, para albergar a fêmea e fecundá-la. Em seguida à ventosa oral, temos o esôfago, que se bifurca ao nível do acetábulo e funde-se depois formando um ceco único que irá terminar na extremidade posterior. Logo atrás do acetábulo, encontramos de sete a nove massas testiculares que se abrem diretamente no canal ginecófaro. O verme não possui órgão copulador e, assim, os espermatozóides passam pelos canais deferentes, que se abrem no poro genital, dentro do canal ginecófaro, daí fecundando a fêmea (Neves, 2005).

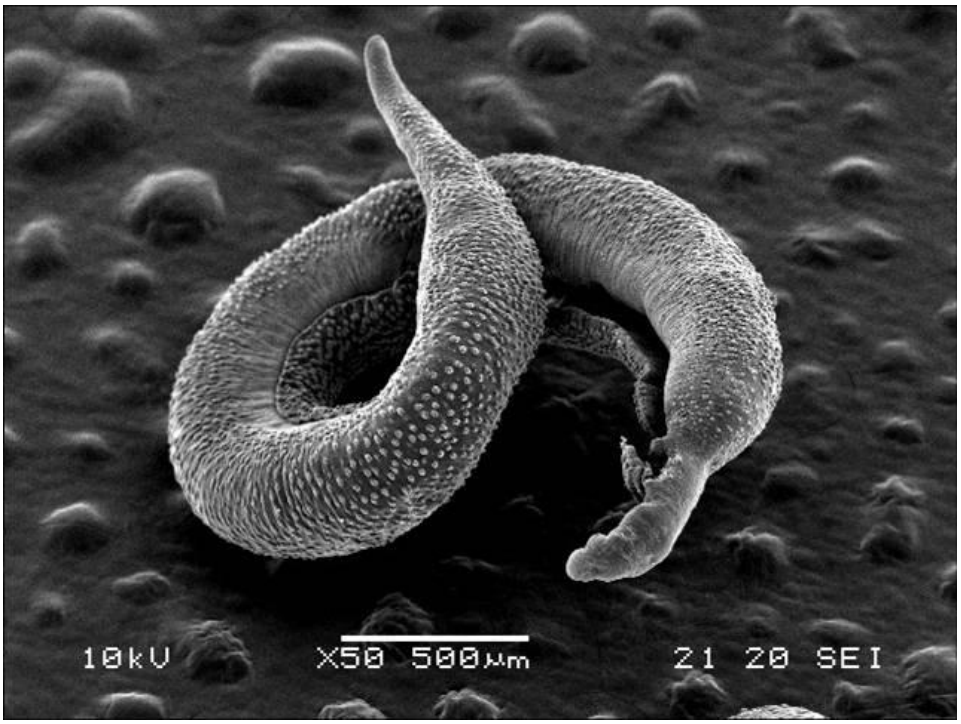


Figura 3: Micrografia Eletrônica de Varredura do Verme Adulto Macho de *Schistosoma mansoni*

Fonte: Pereira et al., 2013.

A fêmea (Figura 4) apresenta comprimento médio de 14mm, corpo filiforme, tegumento liso e mais escuro que o macho, com coloração castanho-escura devido à maior taxa de ingestão e quebra de hemoglobina em hemozoína. Em sua porção anterior encontra-se a ventosa oral e o acetábulo, semelhante ao macho, seguindo-se da vulva, útero (com um ou dois ovos) e ovário. Em sua porção posterior localizam-se as glândulas vitelogênicas e o ceco. Em seu habitat definitivo, a fêmea fecundada começa a ovipor, produzindo cerca de 300 ovos por dia (Schramm & Haas, 2010). O acasal insinua-se nas vênulas mais estreitas da mucosa ou da submucosa do reto, sigmóide e outros segmentos do intestino, enchendo-os de fiadas de ovos produzidos um a um, os quais passam dos locais de ovoposição para a luz intestinal, onde serão expelidos junto às fezes (Rodrigues & Costa, 1987; Oliveira et al., 2000).

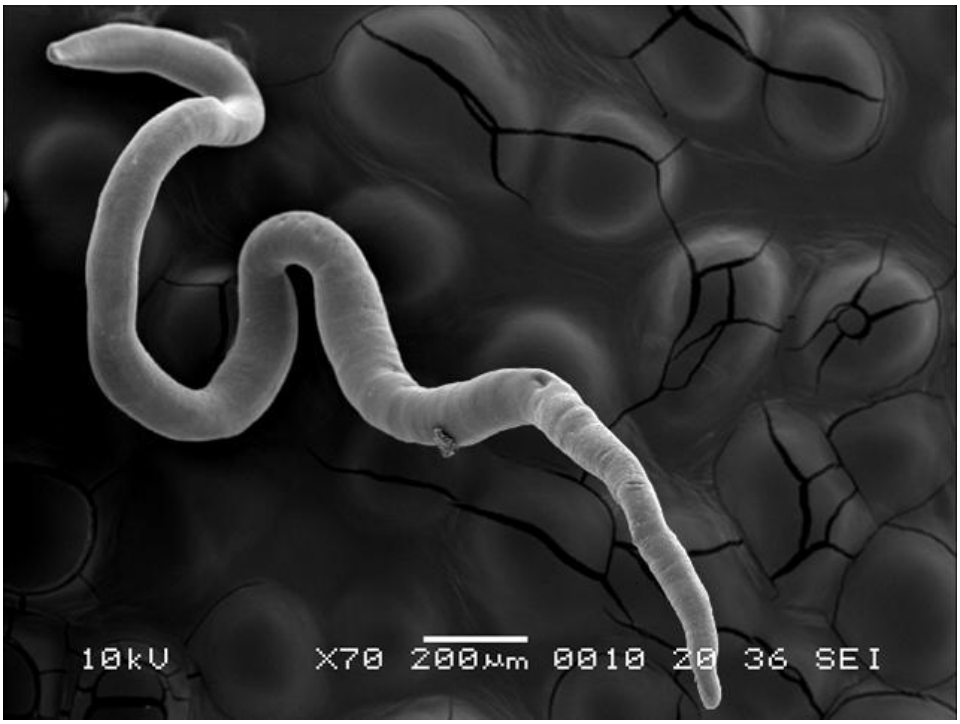


Figura 4: Micrografia Eletrônica de Varredura do Verme Adulto Fêmea de *Schistosoma mansoni*

Fonte: Fonte: Pereira et al., 2013.

3.1.4 Tegumento do Verme adulto do *Schistosoma mansoni*

O tegumento é um complexo sincicial que forma uma interface importante entre o parasita e o hospedeiro, pode apresentar-se como a principal rota para absorção de glicose, aminoácidos e outros nutrientes de forma similar, e também a excreção de produtos metabólicos como acido lático (Cornford & Huot, 1981; Cornford et al., 1983; Bryant, 1993; Camacho & Agnew, 1995).

3.1.4.1 Função do Tegumento

A interação entre moléculas do hospedeiro e o *S. mansoni* pode ser considerada a característica chave para a sobrevivência do parasita. Embora o verme adulto possua função intestinal com a ingestão e digestão de células e macromoléculas, existe um grande tráfego de moléculas pequenas através do seu tegumento. A superfície da membrana do tegumento também liga uma grande variedade de moléculas do hospedeiro, tais como: substâncias dos grupos sanguíneos, antígenos de histocompatibilidade, imunoglobulinas e lipoproteínas (Goldring et al., 1976; Ramalho-Pinto et al., 1978; Sher et al., 1978; Smith & Kusel, 1979; Torpier et al., 1979). Alguns trabalhos têm demonstrado a interação da LDL com cercárias de *S. mansoni*, tal mecanismo de interação prejudicaria o reconhecimento do verme pelo sistema imune do hospedeiro levando a permanência do parasita por longos períodos no organismo infectado (Rumjanek et al., 1983; Bennett & Caulfield, 1991; Tempone et al., 1997; Fan et al., 2003).

3.1.4.2 Morfologia do tegumento

O tegumento do Schistosoma é uma estrutura incomum, sendo envolvida por duas bi-camadas lipídicas muito próximas e opostas na forma de uma membrana plasmática normal que descansa sobre outra região denominada de membranocálice (Wilson et al., 1974; Krautz-Peterson et al., 2007).

Como o tegumento não possui as membranas laterais, seu citoplasma se estende como uma unidade contínua, ou um sincício, ao redor do corpo do verme. O citoplasma tegumental é conectado por numerosos e finos processos citoplasmáticos para

interconectar corpos celulares, que se encontram abaixo das camadas periféricas do músculo; estes contêm núcleos, retículo endoplasmático, complexo de Golgi e mitocôndrias. A importação dos nutrientes através da superfície do tegumento implica a presença de proteínas de transporte (chamadas às vezes de permeases) na membrana plasmática do tegumento (Krautz-Peterson et al., 2007).

O tegumento do *S. mansoni* apresenta numerosas e variadas saliências e organelas tácteis. A presença de poros e fissuras sugere a possibilidade de substratos ou metabólitos entrarem ou saírem através dessas aberturas (Figura 5) (Senft & Gibler, 1977).

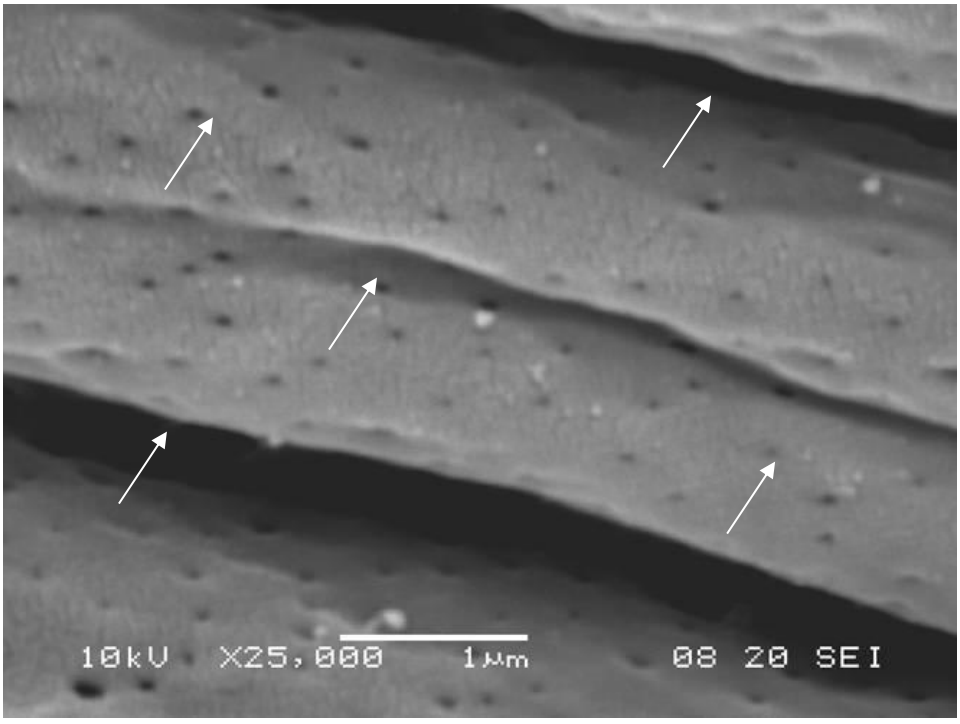


Figura 5: Micrografia de Varredura das dobraduras da região mediana dorsal do verme adulto macho do *Schistosoma mansoni*, apresentando poros no tegumento

Fonte: Pereira et al., 2013.

A região anterior do verme adulto de *S. mansoni*, onde ficam localizadas as ventosas, oral e ventral, consiste numa superfície com algumas espículas emergindo do tegumento e limitada por um número pequeno de poros. Esta região possui dobraduras

menores que outras regiões e é composta por pequenas ranhuras e canais (Gobert et al., 2003). A face dorsal, mediana e posterior do parasita, macho apresenta um aspecto dobrado com numerosos tubérculos recobrindo todo o dorso, desde as ventosas até a cauda.

Uma grande característica topográfica do *S. mansoni* inclui um achatamento dorso-ventral da extremidade posterior que se curva sobre a fêmea látero-ventralmente, formando o canal ginecóforo (Neves, 2005). Na região do canal ginecóforo são observadas muitas aberturas, possivelmente para trocas de substâncias entre o macho e a fêmea, enquanto esta se encontra dentro do canal.

Alguns estudos têm demonstrado que a condição nutricional do hospedeiro definitivo influência os valores morfométricos do verme (Neves et al., 2001). Vermes obtidos de animais com baixa quantidade de proteína apresentam tubérculos mais numerosos, planos, concentrados e áreas vacuoladas. Tais características não são vistas nos animais tratados com alta quantidade de proteína. Não há informações disponíveis a cerca da relação entre a espessura do tegumento e as condições fisiológicas do hospedeiro.

3.1.4.3 Mecanismos de Sobrevivência do Parasito

Os mecanismos da resposta imune nas infecções helmínticas são múltiplos devido ao tamanho e à diversidade metabólica dos parasitos, que são antigenicamente complexos. Um problema adicional é que os parasitos podem sobreviver por muitos anos no hospedeiro, a exemplo do que acontece com o *S. mansoni* (Neva & Brown, 1994). No processo de co-evolução entre parasito-hospedeiro, o schistosoma desenvolveu diversas formas para facilitar sua sobrevivência. Dentre as vantagens que o parasito utiliza para adaptação ao hospedeiro estão: capacidade de modular o sistema imune do hospedeiro como forma de escape, utilizando de mimetismo antigênico, no qual sintetiza moléculas similares ou rouba moléculas do hospedeiro; trocas contínuas do tegumento, recuperando áreas que possam eventualmente ter sido degradadas;

proteases de superfícies que degradam proteínas do complemento e anticorpos; além de poder modular a expressão de proteínas de superfície, que podem ser expressas ou não em determinadas fases do ciclo de vida do parasito (Abath & Werkhauser, 1996; van Hellemond et al., 2006).

Devido à habilidade do parasito de evadir-se dos mecanismos do sistema imune do hospedeiro, o desenvolvimento de uma vacina efetiva contra a esquistossomose permanece difícil (Chen et al., 2002).Os mecanismos moleculares que permitem a estes parasitos permanecerem nos vasos sangüíneos, dando origem a infecções crônicas sem serem destruídos pelo sistema imune do hospedeiro, ainda não são completamente compreendidos (Smith & Kusel, 1979; Torpier et al., 1979; van Balkom et al., 2005). Considerando este cenário, o desenvolvimento de novos tratamentos para a esquistossomose beneficiaria milhões de pessoas que vivem em países em desenvolvimento (Demarco & Verjovski-Almeida, 2009).

3.2 Esquistossomose

A esquistossomose é uma das mais importantes doenças tropicais negligenciadas (van der Werf et al., 2003; Chammartin et al., 2013; WHO, 2013).

3.2.1 Epidemiologia

De ocorrência mundial, esta doença está presente em 77 países afetando mais de 243 milhões de pessoas, com aproximadamente 779 milhões sob risco de infecção em áreas endêmicas (Figura 6) (Engels et al., 2002; van der Werf et al., 2003; Gryseels et al., 2006; Attallah et al., 2008; Allam, 2012; WHO, 2013). É uma doença parasitária crônica e debilitante, que ocupa o segundo lugar, perdendo apenas para a malária em termos de morbidade e mortalidade (Hotez & Fenwick, 2009; Fenwick et al., 2009).

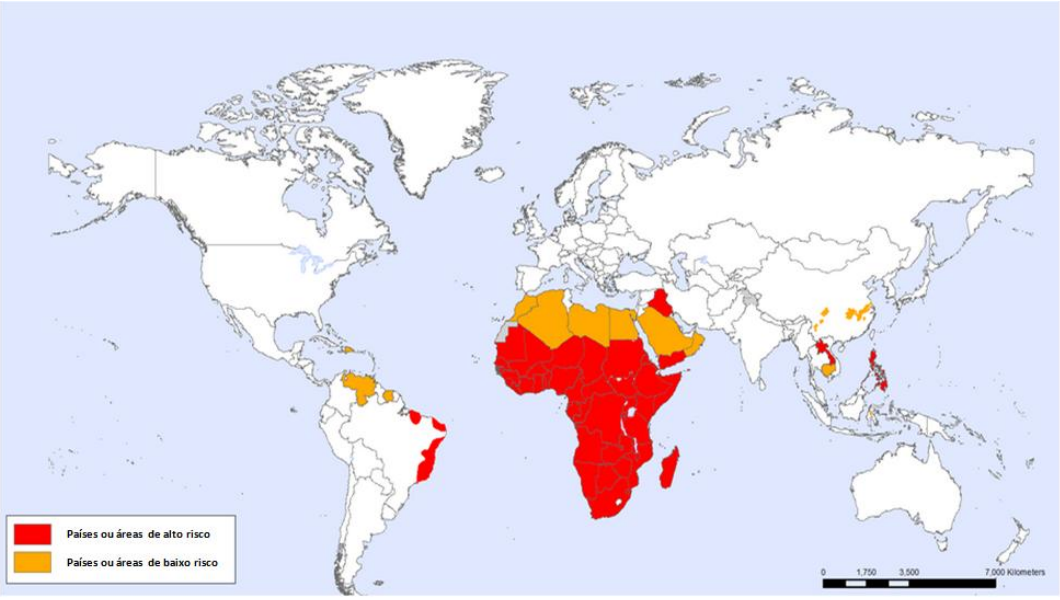


Figura 6: Esquistossomose: Regiões ou Países sob risco em 2011

Fonte: World Health Organization, 2013.

A ocorrência desta parasitose está muitas vezes relacionada à ausência ou precariedade de saneamento básico e condições favoráveis do meio ambiente (Amaral & Porto, 1994; Carmo, 1999; Souza et al., 2007; Chammartin et al., 2013). Por isso a infecção é prevalente em áreas tropicais e subtropicais, em comunidades pobres, sem água potável e saneamento adequado. Vários milhões de pessoas em todo o mundo sofrem de morbidade grave como conseqüência da doença (Ross et al., 2002; van der Werf et al., 2003; King et al. 2005).

Mais de seis milhões de pessoas encontram-se infectadas pelo *S. mansoni* no Brasil e a prevalência está aumentando em 19 estados como exemplos temos: Pará, Piauí, Paraíba, Pernambuco, Alagoas e Sergipe, e uma grande parte da Bahia, São Paulo, Rio de Janeiro, Minas Gerais e do Espírito Santo (Figura 7) (Souza et al., 2007).

Figura 7: Áreas endêmicas e focais da esquistossomose mansonica. Brasil, 2008

Fonte: Brasil, 2009.

Em Pernambuco, a doença é historicamente endêmica na Zona da Mata. Porém, a recente notificação de casos de esquistossomose aguda e de focos da doença no litoral do estado aponta para uma expansão da endemia com mudanças no seu perfil clínico-epidemiológico (Araújo et al., 2006; Barbosa et al. 2012). O estado de um modo geral apresenta condições de saneamento básico precárias. Esse fator aliado a aspectos ligados ao comportamento populacional, a condições ambientais propícias à existência do hospedeiro intermediário, intensa mobilidade das comunidades e à falta de um programa de controle eficaz, concorrem para a criação de condições propícias à manutenção da transmissão e expansão da esquistossomose (Resendes et al., 2005; Leal Neto et al., 2012).

Em Pernambuco foi instalado o Sistema de Informação do Programa de Controle da Esquistossomose (SISPE), o qual foi implantado em 103 municípios, destes, são considerados endêmicos os municípios que tem a presença do caramujo e registram

casos em mais de uma localidade, das Gerências Regionais de Saúde - GERES (I, II, III, IV, V e XII), e localizados no litoral, zona da mata e parte do agreste.

Segundo dados do Sistema de Informação do Programa de Controle da Esquistossomose (SISPCE), em 2012, foram realizados 152.110 exames coproscópicos para esquistossomose no estado. Desses 6.026 (4,0%) foram positivos e 3.880 (64,4%) foram tratados. Os 2.146 casos sem receber tratamento ocorreram por inúmeras situações como: contra-indicações, recusa, ausência por tempo indeterminado, mudança de endereço entre outros. Dentre as GERES de maior índice de positividade, destaca-se a III GERES, com município sede em Palmares, que pertence à zona da mata sul (Pernambuco, 2012). O diagnóstico da esquistossomose é feito por laboratórios do Estado, apresentando 10,90% de prevalência, como mostra a tabela 1.

Tabela 1: Número de exames realizados para diagnóstico da esquistossomose, positividade e proporção de tratados, segundo GERES-PE, 2012.

REGIONAL-PE	EXAMES	POSITIVOS	% POSIT.	TRATADOS	%TRAT.
I GERES	36.001	1.437	4,0	750	52,2
II GERES	30.911	1.251	4,0	641	51,2
III GERES	7.394	804	10,9	677	84,2
IV GERES	26.576	479	1,8	392	81,8
V GERES	29.305	705	2,4	389	55,2
XII GERES	21.923	1.350	6,2	1.031	76,4
Total	152.110	6.026	4,0	3.880	64,4

SISPE/GDNTV/DGCDA/SEVS/SES-PE. Dados obtidos em: 04/03/2013.

Fonte: Pernambuco, 2012.

3.2.2 Descrição da Doença

A esquistossomose é uma infecção transmitida ao homem pelo contato com água de coleções hídricas contaminadas por cercárias, uma das fases do ciclo evolutivo do *S. mansoni* (Ribeiro et al., 2004).

A patogenia da esquistossomose está ligada a vários fatores, tais como linhagem do parasito e a carga parasitária adquirida, idade, estado nutricional, predisposições genéticas e a resposta imune do hospedeiro humano. De todos esses fatores parece que os dois mais importantes são: a carga parasitária e a resposta imune do paciente (Neves, 2005). A esquistossomose mansônica é uma doença de evolução crônica e de gravidade variada. A maioria das pessoas infectadas pode permanecer assintomática, dependendo da intensidade da infecção. A sintomatologia clínica corresponde ao estágio de desenvolvimento do parasito no hospedeiro, a doença caracteriza-se por uma fase aguda e outra crônica quando os vermes adultos, machos e fêmeas, passam a viver nas veias mesentéricas do hospedeiro definitivo (Ribeiro et al., 2004). A doença é causada principalmente por ovos depositados nos tecidos do hospedeiro.

3.2.3 Diagnóstico e Tratamento

O Diagnóstico laboratorial pode ser realizado de 4 formas: *Exame parasitológico de fezes* - Chamado de método Kato-Katz é o mais utilizados devido à sua facilidade operacional em campo e por permitir a quantificação de ovos por grama de fezes; *Biópsia retal* - É um método de fácil execução, porém por ser invasiva, é pouco utilizada, contudo nos casos de esquistossomose crônica, sem hipertensão portal, este método apresenta cerca de 80% de positividade enquanto que no exame de fezes apenas 50%; *Reações sorológicas* - o diagnóstico utilizando soro do paciente para detecção de IgG e IgM contra *S. mansoni* pode indicar infestação atual ou passada. Existem vários tipos de reações sorológicas para a esquistossomose, mas não têm ampla aplicação na prática. *Intradermorreação* - consiste na inoculação de antígeno geralmente preparado com vermes adultos ou cercárias, na face anterior do antebraço. Sua interpretação é feita 15 minutos após a inoculação (Neves, 2005; Brasil, 2009).

Ainda existe o diagnóstico por imagem, onde o uso da ultra-sonografia hepática é de extrema importância no diagnóstico da fibrose de Symmers. Esta fibrose é causada pelos ovos produzidos pelas fêmeas que não são eliminados nas fezes, ficando retidos no intestino e no fígado (Brasil, 2009).

Uma das estratégias para controle da esquistossomose é através da quimioterapia preventiva, controlando a infecção, transmissão e diminuindo a morbidade (Savioli et al., 2004; Silva et al., 2012; WHO, 2013). Estudos mostram que a quimioterapia pode reduzir também a hepatoesplenomegalia. Praziquantel (Cestox®) é a droga de escolha contra todas as espécies, podendo ser utilizado também o oxamniquine (Mansil®). Os efeitos colaterais de ambas as drogas são tonturas, náuseas, cefaléia, sonolência, sendo a tontura mais freqüente com oxamniquine e náuseas e vômitos com praziquantel. O tratamento cirúrgico da esquistossomose está indicado nas formas com complicações, como hipertensão portal e outras (Brasil, 2009).

3.3 Lipoproteínas

Nos animais, os lipídios assumem um papel multifuncional de grande importância. O colesterol e os ácidos graxos desempenham funções essenciais para fluidez das membranas e armazenamento de energia, além de atuarem como moléculas precursoras e sinalizadoras de outras moléculas (Rifai et al., 1999). Para que desempenhem seu papel no metabolismo celular, os lipídios necessitam ser transportados para outros tecidos, porém devido à sua hidrofobicidade, estes utilizam complexos macromoleculares designados lipoproteínas (Lehninger, 2011).

3.3.1 Conceito

As lipoproteínas são partículas globulares com diferentes tamanhos e composições, sintetizadas pelo fígado e intestino. Essas partículas dinâmicas estão em constante estado de síntese, degradação e remoção do plasma (Rader & Hobbs, 2005). Apresentam uma superfície hidrofílica e um núcleo hidrofóbico (Halpern, 1995), sendo classificadas por tamanho e densidade. Podem ser separadas a partir do plasma por ultracentrifugação diferencial (Lalla & Gofman, 1954; Havel et al., 1995; Rifai et al., 1999; Lehninger, 2011).

3.3.2 Classificação, estrutura e função das lipoproteínas

Os principais lipídios transportados por partículas de lipoproteínas são os triacilgliceróis e o colesterol (livre ou esterificado), obtidos da dieta ou da síntese *de novo* (Stryer, 2004). As lipoproteínas são compostas de um centro de lipídio neutro (contendo triacilglicerol ou ésteres de colesterol, ou ambos) circundando por uma concha de apolipoproteinas (apoproteínas), fosfolipídeo e colesterol não-esterificado, todos orientados de modo que suas porções polares estejam expostas na superfície da lipoproteína, tornando assim a partícula solúvel em meio aquoso (Figura 8) (Lehninger, 2011).

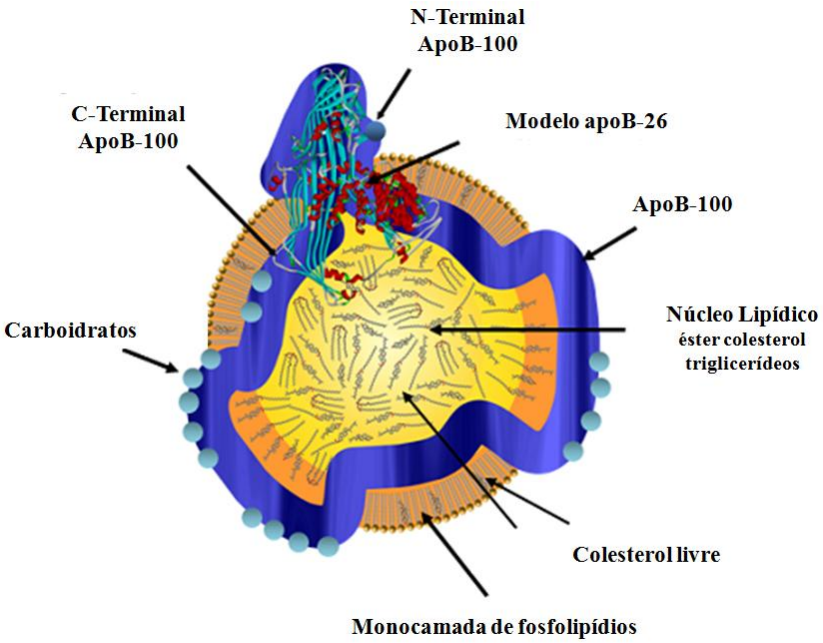


Figura 8: Estrutura de uma Proteína de Baixa Densidade (LDL)

Fonte: Prassl & Laggner, 2009.

As lipoproteínas variam significativamente em tamanho, densidade e composição, o que permite a sua categorização em classes distintas (Figura 9). A densidade destas partículas varia em relação inversa com o seu tamanho, em conseqüência dos níveis de lipídios não polares de baixa densidade presentes no centro e das proteínas na superfície. Assim, as partículas de lipoprotéicas podem ser classificadas como: quilomícrons (QM), lipoproteínas de muito baixa densidade

(VLDL), lipoproteínas de baixa densidade (LDL) e lipoproteínas de alta densidade (HDL) (Havel et al., 1995; Rifai et al., 1999; Stryer, 2004).

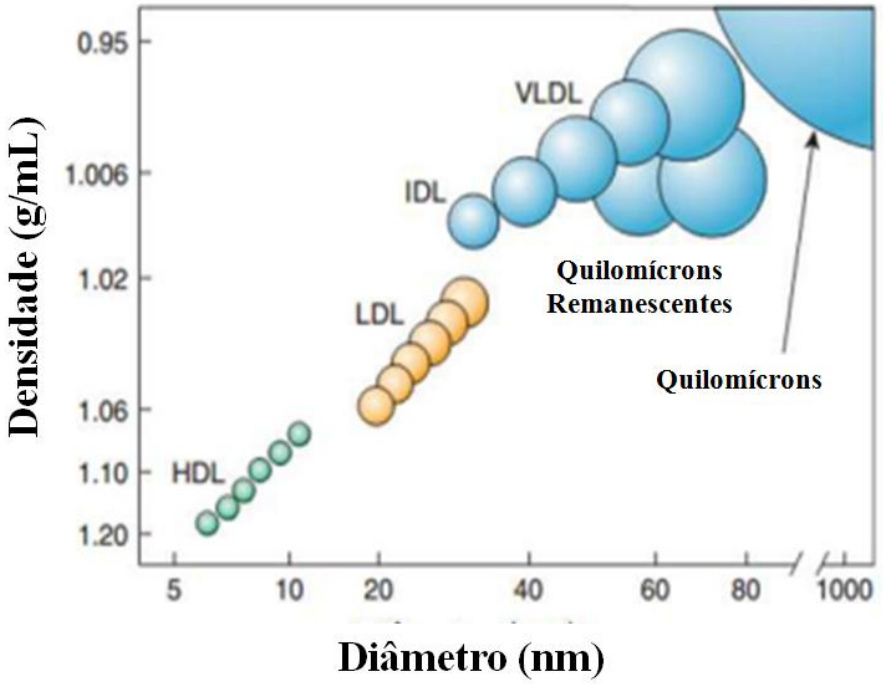


Figura 9: Distribuição da densidade e tamanho das lipoproteínas

Fonte: Rader & Hobbs, 2005.

Cada classe de lipoproteína tem uma função especifica determinada por seu lugar de síntese, composição lipídica e conteúdo de apopoliproteína. As apoproteínas controlam o metabolismo das lipoproteínas ligando-se em receptores de membrana específicos. Elas agem como co-fatores para enzimas que participam do metabolismo das lipoproteínas, solubilizando os lipídios hidrofóbicos, conferindo assim integridade estrutural às partículas (Rifai et al., 1999; Stryer, 2004; Pamella, 2005).

Pelo menos nove apopoliproteínas diferentes são encontradas entre as lipoproteínas do plasma humano (ApoA-I, ApoA-II, ApoA-IV, ApoB-48, ApoB-100, ApoC-I, ApoC-II, ApoD e ApoE.); elas podem ser distinguidas por seu tamanho, suas

reações com anticorpos específicos e sua distribuição característica nas classes de lipoproteínas (Lehningher, 2011).

Os quilomícra são partículas de lipoproteína de menor densidade e maior tamanho, são sintetizados pelas células intestinais e sua apolipoproteína principal é a apoB-48. A VLDL é sintetizada no fígado e sua principal função é o transporte para o plasma sanguíneo dos triglicerídeos produzidos no fígado. A VLDL possui as seguintes apolipoproteínas: apoB-100, apoE e apoC. Quando em contato com a enzima lipase protéica as VLDL dão origem as IDLs. Estas partículas são produtos intermediários que em contato com a lipase hepática formam a LDL (Rader & Hobbs, 2005). Na LDL a única proteína presente é a apoB-100, localizada em torno de sua superfície, estabilizando a estrutura do complexo proteína-lipídio. Sua principal função é controlar o metabolismo da lipoproteína ligando-se em receptores de membrana específicos. É altamente insolúvel em solução aquosa, constituída por 4.536 resíduos de aminoácidos, apresenta massa molecular de 550 kDa para a forma glicosilada (Segrest et al., 2001). A lipoproteína mais abundante no plasma e principal transportadora de colesterol é a LDL (Hevonoja et al., 2000). As HDLs são as mais densas lipoproteínas plasmáticas. Sintetizadas no fígado e intestino, possuem como principal componente protéico a apolipoproteína A (A-I; A-II; e A-IV), mas também podem conter apoC e apoE (Castelli et al., 1977).

As lipoproteínas funcionam tanto para manter os lipídios solúveis, à medida que os transporta no plasma, quanto para fornecer um mecanismo eficiente para entrega do conteúdo lipídico aos tecidos. Nos seres humanos, o sistema de entrega é menos perfeito que em outros animais e, como resultado, ocorre uma deposição gradual de lipídios – especialmente colesterol – nos tecidos (Pamella, 2005). Este colesterol em excesso presente na forma de partículas de LDL é chamado de “mau colesterol”. A relação entre o colesterol na forma de HDL e na forma de LDL pode ser usada para avaliar a suscetibilidade ao desenvolvimento de doença cardíaca (Rifai et al, 1999; Stryer, 2004). Representa um risco de vida em potencial quando a deposição de lipídios contribui para a formação de placas, causando o estreitamento dos vasos sanguíneos – uma condição conhecida como aterosclerose.

3.3.3 Metabolismo das Lipoproteínas de Baixa Densidade

O brotamento e fusão de membranas são à base de diversos processos biológicos importantes. As membranas têm que ser capazes de se separar e reunir para captar, transportar e liberar moléculas. Muitas captam moléculas pelo processo de endocitose por receptor, onde uma proteína ou complexo maior liga-se inicialmente a um receptor na superfície da célula. Em seguida, proteínas especializadas agem fazendo com que a membrana invagine na vizinhança da proteína ligada. A membrana invaginada rompe e se funde, formando uma vesícula (Stryer, 2004).

A endocitose por receptor exerce um papel importante no metabolismo do colesterol (Figura 10). As LDLs são removidas do plasma por intermédio de uma glicoproteína de superfície específica, o receptor de LDL (LDLR, *low density lipoprotein receptor*). O LDLR tem a capacidade de reconhecer dois ligantes: a apoB e a apoE (Goldstein et al., 1995). Na membrana celular, os receptores de LDL localizam-se preferencialmente em "coated pits", regiões especializadas da membrana cobertas por clatrina na superfície interior.

Figura 10: Receptor de LDL associado ao revestimento de clatrina no processo de endocitose

Fonte: http://dc147.4shared.com/doc/mqtuPQXO/preview.html

(Endereço eletrônico acessado dia 24 de janeiro de 2014)

Quando se liga uma partícula de LDL ao receptor, por reconhecimento da apoB-100 na sua superfície, a clatrina polimeriza e forma invaginações na membrana que posteriormente, originam vesículas revestidas de clatrina com os complexos LDLR–LDL no seu interior (Goldstein et al., 1995). Para que ocorra internalização do LDLR é necessária, na maioria dos tecidos, uma proteína adaptadora que se liga à cauda citoplasmática do receptor, a LDLRAP1 (LDL *receptor adaptor protein 1*) (Norman et al., 1999). As proteínas adaptadoras são necessárias, mas não suficientes para montagem do revestimento de clatrina das vesículas. De fato, diversas proteínas acessórias, como a chaperona de choque térmico (HSP 70) têm sido descritas na endocitose mediada por clatrina, desempenhando papel fundamental na regulação da via e no transporte de moléculas dentro da célula (Lemon, 2001; Conner & Schmid, 2003).

As vesículas de clatrina que transportam os complexos LDLR–ligante vão fundir-se no citoplasma e formar endossomos onde, por diminuição do pH, o receptor se dissociadas partículas de LDL (Davis et al., 1987). O receptor é direcionado para vesículas de reciclagem, que fazem o seu retorno à membrana celular para realizar novo ciclo e as partículas de LDL vão ser degradadas nos lisossomos (Gillian-Daniel et al., 2002). Cada ciclo de atividade do LDLR tem a duração aproximada de 10 minutos (Goldstein et al., 1995). O colesterol é liberado na célula, para armazenamento ou uso na biossíntese de membranas, e os componentes protéicos restantes são degradados. Vários hormônios, proteínas de transporte e anticorpos empregam endocitose por receptor para entrada na célula. Uma conseqüência menos vantajosa é que esta via está disponível para vírus e toxinas como um meio de entrada nas células (Bennet & Caulfield, 1991; Xu et al., 1993; Stryer, 2004).

3.3.4 Papel da Hsp 70 na endocitose mediada por clatrina

O tipo mais freqüente, e provavelmente o melhor caracterizado, de endocitose envolve vesículas revestidas pela proteína clatrina, que atuam na internalização de receptores e seus ligantes. Muitos dos ligantes são posteriormente degradados em endosomos tardios ou lisosomos, enquanto os receptores são reciclados para a

membrana plasmática e reutilizados até várias centenas de vezes (Maxfield & McGraw, 2004).

Na endocitose mediada por clatrina, o material entra na célula em vesículas formadas por invaginações e subsequente brotamento de porções da membrana plasmática. Vesículas revestidas de clatrina são encontradas em todas as células nucleadas (Conner & Schmid, 2003). Este tipo de endocitose tem um papel importante na internalização de lipídios, proteínas e macromoléculas, na reciclagem de vesículas sinápticas e na regulação de receptores de superfície (Young, 2007). Além disto, vesículas revestidas com clatrina também estão envolvidas na via secretória, atuando no transporte de glicoconjugados a partir da rede trans-Golgi (TGN) ao endosomo tardio ou à membrana plasmática (Brodsky et al., 2001; Kirchhausen, 2000).

Na primeira descrição de endocitose mediada por receptor foi observado que partículas de LDL ligavam-se às células, eram agregadas em vesículas revestidas e então internalizadas (Goldstein et al., 1979). Assim, anos antes do receptor para LDL ser purificado, esse estudo sugeriu que receptores de superfície celular para LDL e outros ligantes poderiam ser proteínas transmembranares com um sítio citoplasmático para ligação de elementos de formação de revestimento de vesículas de endocitose (Roth, 2006).

O estudo da endocitose mediada por clatrina foi iniciada pela análise de vesículas revestidas que formavam uma gaiola regular com estrutura composta por pentágonos e hexágonos (Kanaseki & Kadota, 1969; Roth, 2006). Posteriormente outros pesquisadores purificaram vesículas e mostraram que esse revestimento era composto primariamente por uma proteína, à qual foi chamada de clatrina por seu aspecto semelhante a uma grade (clatratus, em latim). Também foi determinada a estrutura com que a clatrina se polimerizava, formando um trímero, que foi chamado de “trisquélion” (Pearse, 1976). Esse trímero de clatrina não se liga diretamente à membrana plasmática, mas necessita de proteínas acessórias que se posicionem entre a clatrina e a membrana, chamadas hoje de proteínas adaptadoras (Pearse, 1987; Fotin et al., 2004).

Nos últimos anos a compreensão dos detalhes moleculares da endocitose mediada por clatrina tem aumentado enormemente. Muitas proteínas foram descobertas que funcionam como adaptadores carga-específicos que trazem os complexos receptores-ligantes para os sítios de membrana com revestimento (Roth, 2006).

As Hsps são conhecidas como proteínas de choque térmico, elas formam o sistema de defesa mais antigo em todos os organismos vivos da Terra e são de fundamental importância para a sobrevivência das células. Esta família de proteínas não se apresenta na célula apenas em condição de estresse, mas também no dobramento das proteínas, na interação com receptores de esteróides e no transporte de proteínas através das membranas de algumas organelas celulares (Craig, 1985; Gething & Sambrook, 1992; Morimoto, 1993; Terlecky, 1994; Gabriel et al., 2002).

A Hsp70 demonstra uma importante capacidade de ligação a uma grande variedade de proteínas recentemente sintetizadas (Beckman et al., 1990). Assim, pode se ligar a proteínas, independentemente da estrutura, apenas pelo reconhecimento de regiões de caráter hidrofóbico. A Hsp70 é difusamente localizada no citoplasma e no núcleo (Welch & Feramisco, 1984). A síntese das Hsps ocorre rapidamente após a exposição do organismo a estresses externo, térmicos ou não, com o objetivo de manter a homeostase celular e a conformação das proteínas intracelulares, bem como evitar a desnaturação e a má-formação de proteínas durante sua síntese, além de proverem a organização, translocação e ação das chaperonas, acompanhantes de proteínas até seus sítios definitivos (Fehrenbach & Niess, 1999).

A Hsp70 está presente em várias formas de vida do *S. mansoni* (miracidio, esquistossômulo e verme adulto) e sugere-se que ela esteja envolvida no processo de internalização de LDL no parasita (Levy-Holtzman & Schechter, 1996, Kanehisa, 2000). A figura 11 apresenta um dos papeis desempenhado pela da Hsp70 no processo de encocitose dentro da célula (Lemmon, 2001).

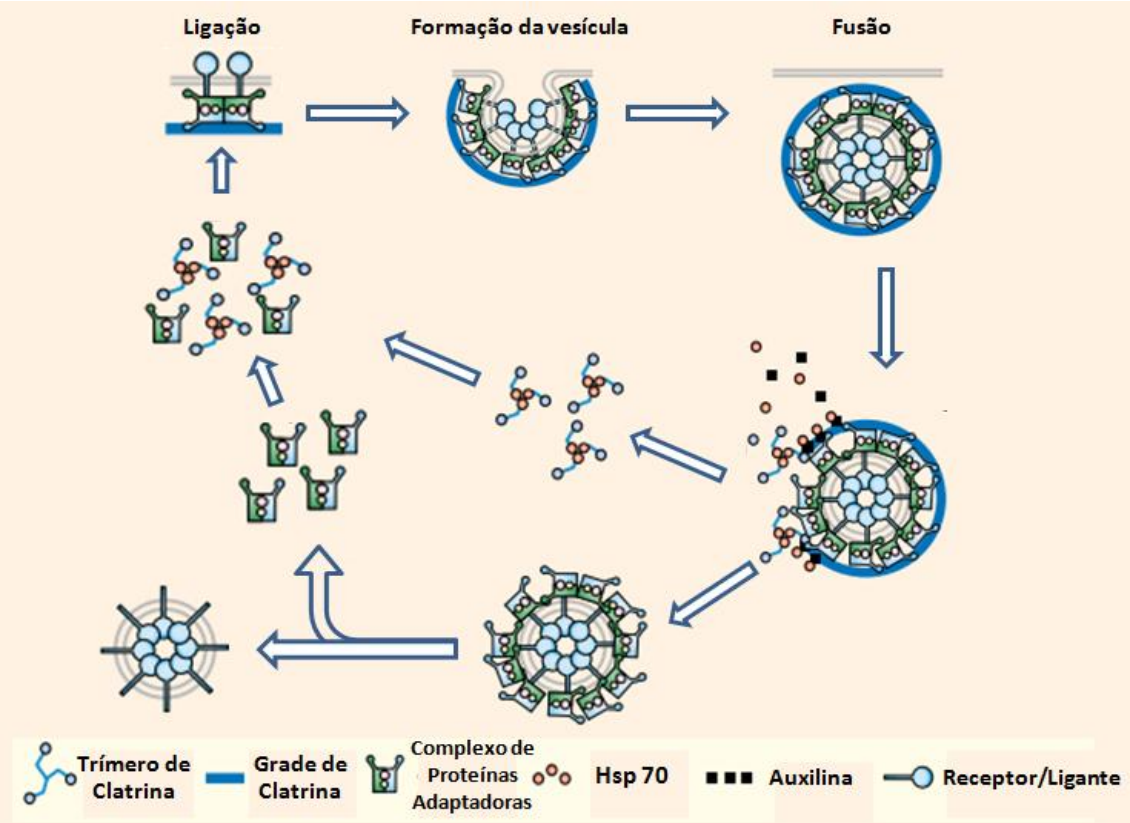


Figura 11: Ciclo da endocitose mediada por clatrina

Fonte: Lemmon, 2001.

3.3.5 Receptores de lipoproteínas no *Schistosoma*

As lipoproteínas do plasma são moléculas de interesse particular, pois apresentam uma fonte rica e abundante de colesterol e outros lipídios que podem ser usados por parasitas que habitam o sistema vascular dos hospedeiros (Rogers, 1991). Muitos deles não são capazes de sintetizar os ácidos graxos ou colesterol de cadeia longa pela via *de novo*, e estas moléculas são importantes para manter a integridade da superfície da membrana, que forma a interface entre o parasita e o hospedeiro (MacGregor et al., 1989; Rogers, 1991). Já foi discutida a presença de receptores de lipoproteína em vários parasitas como: *Trichomonas vaginalis*, *Trypanosoma brucei*, *Trypanosoma cruzi*, *Plasmodium falciparum*, além do Schistosoma (Peterson & Alderete, 1984; Rumjanek et al., 1985; Coppens et al., 1987; Prioli et al., 1988; Rogers

et al., 1989, Grellier et al., 1991; Fan et al., 2003). Existem evidências que sugerem que estes parasitas expressem receptores para lipoproteínas em suas membranas, sendo estes componentes cruciais para a aquisição de lipídios.

A lipoproteína de baixa densidade (LDL), o maior carreador de colesterol no plasma humano, se liga à superfície da larva e dos vermes adultos de Schistosoma (Fan et al., 2003). Em trabalhos realizados com esquistossômulo de *S. mansoni*, a forma larval que primeiro estabelece contato com o interior do hospedeiro definitivo, observou-se que eles expressavam uma proteína de 45 kDa (Rumjanek et al., 1988) assim que entravam em contato com o soro humano e que, quando a LDL humana era adicionada ao meio tornava-se adsorvida sobre sua superfície (Rumjanek et al., 1985). Receptores ou proteínas ligantes envolvidas na interação com LDL ou VLDL têm sido descritos em esquistossômulos (Rumjanek, 1985, 1988; Chiang & Cauldfield, 1989) e vermes adultos de *S. mansoni* (Tempone et al.,1997) e *S. japonicum* (Fan et al., 2003).

Em trabalhos realizados com vermes adultos de *S. japonicum* foi observada a ocorrência de um suposto receptor para LDL humana, com um peso molecular aparente de 43 kDa, o qual esteve presente no tegumento e no intestino dos parasitas, e cuja interação com a lipoproteina requer a presença de lisina e arginina (Rogers et al., 1989; Rogers et al., 1990).

A LDL é rica em esteróis e fosfatidilcolina, necessários para síntese de membranas, podendo funcionar como fonte de lipídios para o parasita e ao mesmo tempo, como proteção contra os ataques do sistema imune do hospedeiro, mascarando antígenos do parasita. (Chiang & Caulfield, 1989).

3.4 Schistosoma e Proteômica

A proteômica é uma poderosa ferramenta utilizada para detectar e identificar as proteínas em amostras específicas provenientes de schistosomas, representando uma abordagem interessante para investigar e fornecer novas perspectivas para a biologia celular do parasito. Até agora, a maioria dos estudos proteômicos neste organismo têm sido principalmente descritivos, com o objetivo de identificar as proteínas mais

abundantes em uma fração específica derivada de uma ou mais etapas do ciclo de vida do helminto. Estas proteínas abundantes são obviamente importantes, mas não necessariamente desempenham funções essenciais na biologia específica do esquistossomo, tais como a interação com o hospedeiro. Assim, determinadas sub-frações, que são conhecidas por desempenhar funções específicas, precisam ser purificadas a fim de identificar proteínas menos abundantes, mas funcionalmente importantes. A este respeito, os estudos mais recentes sobre proteoma do parasito estão focados na identificação de proteínas, em especial em frações purificadas, tais como os produtos de excreção e secreção (Curwen et al., 2006; Perez-Sanchez et al., 2006), proteínas específicas do tegumento (Van Balkom et al., 2005) ou proteínas de superfície exposta em vermes adultos (Braschi & Wilson, 2006). Estudos proteômicos têm dessa forma gerado uma lista mais completa de proteínas envolvidas em funções específicas do Schistosoma (Van Hellemond et al., 2006).

O principal desafio na investigação da biologia deste parasito continua sendo a elucidação da função de proteínas específicas fundamentais para desvendar fatos intrigantes da sua biologia como, por exemplo, sua interação com o hospedeiro.

REFERÊNCIAS

Abath, F.G.C., Werkhauser, R.C. The tegument of *Schistosoma mansoni:* functional and immunological features. **Parasite Immunology**, v.18, p. 15-20, 1996.

Acton, S.L., Scherer, P.E., Lodish, H.F., Krieger, M. Expression cloning of SR-BI, a CD36-related class B scavenger receptor. **Journal of Biological Chemistry**, v.269, p.21003-21009, 1994.

Allam, A.F. **Schistosomiasis**. Chapter 1, Diagnosis of Schistosomiasis in Low Endemic Areas. Edited by Mohammad Bagher Rokni, January, 2012 ISBN 978-953-307-852-6, 2012.

Amaral, R.S., Porto, M.A.S. Evolução e situação atual da Esquistossomose no Brasil. **Revista da Sociedade Brasileira de Medicina Tropical**, v. 27, p.73- 90, 1994.

Araújo, K.C., Resendes, A.P., Souza-Santos, R. Análise espacial dos focos de *Biomphalaria glabrata* e de casos humanos de esquistossomose mansônica em Porto de Galinhas, Pernambuco, Brasil, no ano 2000. **Cadernos de Saúde Pública**, v. 23 (2), p. 409-417, 2006.

Attallah, A.M., Wahba, M.A., Elsheikha, H.M., Abbas, A.T., Aziz, A.M.M., El-Hemaly, M.A. Outcomes of *Schistosoma mansoni* infection in outbred albino mice exposed to Larvin contaminant. **Parasitology Research**, v.103, p.567-576, 2008.

Barbosa, C.S.; Barbosa, F.S. Padrão epidemiológico da esquistossomose em comunidade de pequenos produtores rurais de Pernambuco, Brasil. **Cadernos de Saúde Pública**, v. 14(1), p.129-137, 1998.

Barbosa, C. S., Domingues, A. L. C., Abath, A. Epidemia de esquistossomose aguda na praia de Porto de Galinhas, Pernambuco, Brasil. **Cadernos de Saúde Pública**, v.17(3), p. 725-728, 2001.

Barbosa, V.S., Araújo, K.C., Leal Neto, O.B., Barbosa, C.S. Spatial distribution of schistosomiasis and geohelminthiasis cases in the rural areas of Pernambuco, Brazil **Revista da Sociedade Brasileira de Medicina Tropical,** v. 45(5), p. 633-638, Sep-Oct, 2012.

Beckman, R.P., Mizzen, L.A., Welch, W.J. Interaction of hsp70 with newly synthesized proteins: implications for protein folding and assembly. **Science**, v. 248, p.850-854, 1990.

Bennet, M.W., Caulfield, J. P. Specific binding of human low-density lipoprotein to the surface of Schistosoma mansoni and ingestion by the parasite. **American Journal of Pathology**, v.138 (5), p. 1173-1182, 1991.

Braschi, S., Wilson, R. A**.** Proteins exposed at the adult Schistosome surface revealed by biotinylation. **Molecular & Cellular Proteomics,** v. 5, p. 347-356, 2006.

Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. **Guia de vigilância Epidemiológica**, 6 ed. Brasília, Brasil, p. 297–298, 2005.

Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. **Vigilância e controle de moluscos de importância epidemiológica**, 2ª ed - Brasília: Editora do Ministério da Saúde, 2007.

Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. **Guia de Vigilância Epidemiológica**. Brasília. 7ª Ed., Caderno 10, p.24, 2009.

Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Sistema Nacional de Vigilância em Saúde, **Relatório de Situação**, Pernambuco, 5rd edn Brasília, Brasil, p.7, 2011.

Brodsky, F.M., Chen, C.Y., Knuehl C., Towler, M.C., Wakeham, D.E. Biological basket weaving: Formation and Function of Clathrin-Coated Vesicles. **The Annual Review of Cell and Developmental Biology,** v.17, p.517-568, 2001.

Bryant, C. Organic acid excretion by helmints. **Parasitology Today**, v. 9 (2), p. 58-60, 1993.

Camacho, M., Agnew A. *Schistosoma*: Rate of glucose import is altered by acetylcholine interaction with tegumental acetylcholine receptors and acetylcholinesterase. **Experimental Parasitology**, v. 81, p.584-591, 1995.

Carmo, E.H. Morbidade e Mortalidade por Esquistossomose mansônica na Região Nordeste do Brasil. Tese de Doutorado, **Instituto de Saúde Coletiva**, Universidade Federal da Bahia, Salvador, 1999.

Castelli W.P. et al. HDL cholesterol and lipids in coronary heart disease. The cooperative lipoprotein phenotyping study. **Circulation**, v. 55(5), p.767-772, 1977.

Chammartin, F., Hürlimanna, E, Raso, G., N'Goranc, E.K., Utzingera, J., Vounatsou, P. Statistical methodological issues in mapping historical schistosomiasis survey data. **Acta Tropica,** doi: 10.1016/j.actatropica.2013.04.012, 2013.

Chen, L., Rao, K., He, Y. Skin-stage schistosomula of *Schistosoma mansoni* produce an apoptosis-inducing factor that can cause apoptosis of T cells. **The Journal of Biological Chemistry**, v. 277, n. 37 (13), p. 34329–34335, 2002.

Chiang, C., Cauldfield, J.P. Human lipoprotein biding to Schistosomula of *Schistosoma mansoni*. **American Journal of Pathology,** v.135, p.1015-1024, 1989.

Clerinx, J., Van Gompel, A. Schistosomiasis in travellers and migrants. **Travel Medicine and Infectious Disease**, v. 9, p. 6-24, 2011.

Conner, S.D., Schmid, S.L. Regulated portals of entry into the cell. **Nature, v.** 422, p.37-44, 2003.

Coppens, I., Opperdoes, F., Courtoy, P.J., Baldhuin, P. Receptor mediated endocytosis in the bloodstream form of *Trypanosoma brucei*. **Journal of Protozoology**, v.34, p.465-473, 1987.

Cornford, E. M., Diep C. P., Rowley, G. A. *Schistosoma mansoni, S. japonicum, S haematobium*: Glycogen content and glucose uptake in parasites from fasted and control hosts. **Experimental Parasitology**, v. 56, p.397-408, 1983.

Cornford, E.M., Huot, M.E. Glucose transfer from male to female schistosomes. **Science.** v. 213, p. 1269-1271, 1981.

Craig, E.A. The heat shock response. **Critical Reviews in Biochemistry**, v. 18, p. 239-280, 1985.

Curwen, R.S., Ashton, P.D., Sundaralingam, S., Wilson, R.A. Identification of novel proteases and immunomodulators in the secretions of schistosome cercariae that facilitate host entry. **Molecular & Cellular Proteomics**, v. 5, p.835-844, 2006.

Davis, C.G., Goldstein, J.L., Südhof, T.C., Anderson, R.G., Russell, D.W., Brown, M.S. Acid-dependent ligand dissociation and recycling of LDL receptor mediated by growth factor homology region. **Nature**, v. 326, p.760-765, 1987.

Delcroix, M., Medzihradsky, K., Caffrey, C.R., Fetter, R.D., McKerrow, J.H. Proteomic analysis of adult *S. mansoni* gut contents. **Molecular & Biochemical Parasitology**, v. 154, p. 95–97, 2007.

Demarco, R., Verjovski-Almeida, S. Schistosomes- proteomics studies for potencial novel vaccines and drugs targets**. Drug Discovery Today**, v.14, p. 9-10, 2009.

Dorsey, C. H. et al. Ultrastructure of the Schistosoma mansoni cercaria. **Micron**, v. 33, n. 3, p.279-323, 2002.

Engels, D., Chitsulo, L., Montresor, A., Savioli, L. The global epidemiological situation of schistosomiasis and new approaches to control and research. **Acta Tropica**, v.82, p.139-146, 2002.

Fan, J., Gan, X., Yang, W., Shen, L., McManus, D. P., Brindley, P.J. A *Schistosoma japonicum* very low-density lipoprotein-binding protein. **The International Journal of Biochemistry & Cell Biology**, v. 35, p.1436-1451, 2003.

Fehrenbach, E., Niess, A. Role of heat shock protein in the exercise response. **Exercise Immunology Review**, v.5, p.57-77, 1999.

Fenwick, A., Webster, J. P., Bosque-Oliva, E., Blair, L., Fleming, F. M., Zhang, Y., Garba, A., Stothard, J. R., Gabrielli, A. F., Clements, A. C., Kabatereine, N. B., Toure, S., Dembele, R., Nyandindi, U., Mwansa, J. & Koukounari, A. The Schistosomiasis Control Initiative (SCI): rationale, development and implementation from 2002-2008. **Parasitology**, v.136, p.1719-1730, 2009.

Fotin, A., Cheng, Y., Sliz, P., Grigorieff, N., Harrison, S.C., Kirchhausen, T., Walz, T. Molecular model for a complete clathrin lattice from electron cryomicroscopy. **Nature**, v.432, p.573-579, 2004.

Frydman, J., Nimmesgern, E., Ohtsusa, K., Hartl, F. U. Folding of nascent polypeptide chains in a high molecular mass assembly with molecular chaperones. **Nature**, v.370, p.111–117, 1994.

FUNASA. Fundação Nacional de Saúde – **Guia de Vigilância Epidemiológica**, v. 1, 2002.

Gabriel, J.E., Mota, A.F., Boleli, I.C., Macari, M., Coutinho, L.L. Effect of moderate and severe heat stress on avian embryonic hsp70 gene expression. **Growth, Development & Aging**, v. 66, p. 27-33, 2002.

Gething, M. J., Sambrook, J. Protein folding in the cell. **Nature**, v. 355, p. 33-45, 1992.

Gillian-Daniel, D.L., Bates, P.W., Tebon, A., Attie, A.D. Endoplasmatic reticulum localization of the low density lipoprotein receptor mediates presecretory degradation of apolipoprotein B. **Proceedings of the National Academy of Sciences**, v.99, p.4337-4342, 2002.

Gobert, G.N., Stenzel D.J., McManus, D.P., Jones, M.K. The ultrastructural architecture of the adult *Schistosoma japonicum* tegument. **International Journal for Parasitololy**, v. 33, p. 1561-1575, 2003.

Goldring O. L., Clegg, J. A., Smithers, S. R., Terry, R. J. Acquisition of human blood group antigens by *Schistosoma mansoni*. **Clinical and Experimental Immunology**, v.26, p. 181-187, 1976.

Goldstein, J.L., Anderson, R.G., Brown, M.S. Coated pits, coated vesicles, and receptor-mediated endocytosis. **Nature**, v.279, p. 679-685, 1979.

Goldstein, J.L., Hobbs, H., Brown, M.S. Familial Hypercholesterolemia. **The metabolic and molecular bases of inherited disease**. 7ed. New York: McGraw-Hill; p1981-2030, 1995.

Grellier, P., Rigomier, D., Clayey, V., Fruehart, J-C., Sehrevel, J. Lipid Traffic between High Density Lipoproteins and *Plasmodium falciparum* infected Red Blood Cells. The **Journal of Cell Biology**, v.112(2), p.267-277, 1991.

Gryseels, B., Polman, K., Clerinx, J., Kestens, L. Human schistosomiasis. **Institute for Tropical Medicine**, v. 368, p. 1106-18, 2006.

Halpern, M.J. Lipids and atherosclerosis, **Molecular Aspects of Medicine**, v.16(6), p.509-710. 1995.

Han, Z.G., Brindley, P.J., Wang, S.Y., Chen, Z. Schistosoma Genomics: New Perspectives on Schistosome Biology and Host-Parasite Interaction. **Annual Review of Genomics and Human Genetics**, v. 10, p. 211-240, 2009.

Havel, R.J., Eder, H.A., Bragdan, J.H. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. **The Journal of Clinical Investigation**, v. 4, p.1345-1349, 1995.

Hendrick, J. P., Hartl, F. U. Molecular chaperone functions of heat-shock proteins. **The Annual Review of Biochemistry**, v. 62, p. 349-384, 1993.

Hevonoja, T., Pentikäinem, M.O., Hyvönem, M.T., Kovanen, P.T., Ala-Korpela, M. Structure of low density lipoprotein (LDL) particles: Basis for understanding molecular changes in modified LDL. **Biochimica et biophysica Acta (BBA) – Molecular and Cell Biology of lipids**, v. 1488 (3), p. 189-210, 2000.

Hockley, D. J. Schistosoma mansoni: the development of the cercarial tegument. **Parasitology**, London, v. 64 (2), p. 245-52, Apr. 1972.

Holtzman, R.L., Schechter, I. Expression of different forms of the heat-shock factor during the life cycle of the parasitic helminth *Schistosoma mansoni.* **Biochimica et Biophysica Acta**, v.1317, p.1-4, 1996.

Hotez, P.J., Fenwick, A. Schistosomiasis in Africa: An Emerging Tragedy in Our New Global Health Decade. **PLOS Neglected Tropical Diseases**, v.3(9), e485, p. 1-3, 2009.

Jones, M. K., Gobert, G. N., Zhang, L., Sunderland, P., McManus, D. P. The cytoskeleton and motor proteins of human schistosomes and their roles in surface maintenance and host–parasite interactions. **BioEssays**, v.26, p. 752-765, 2004.

Kanaseki, T., Kadota, K. The "vesicle in a basket". A morphological study of the coated vesicle isolated from the nerve endings of the guinea pig brain, with special reference to the mechanism of membrane movements**. The Journal of Cell Biology**, v.42, p.202-220, 1969.

Kanehisa, M. **Post-genome Informatics**, Oxford University Press. 2000.

Katz, N., Peixoto, S. V. Análise crítica da estimativa do número de portadores de esquistossomose mansônica no Brasil. **Revista de Sociedade Brasileira de Medicina Tropical**, São Paulo, v. 33, n.3, 2000.

King, C.H., Dickman, K., Tisch, D.J. Reassessment of the cost of chronic helmintic infection: a meta-analysis of disability-related outcomes in endemic schistosomiasis. **Lancenet**, v. 365, p. 1561-1569, 2005.

Kirchhausen, T. Clathrin. **The Annual Review of Biochemistry**, v.69, p.699-727, 2000.

Krautz-Peterson, G., Camargo, S., Huggel, K., Verrey, F., Shoemaker, C.B., Skelly, P.J. Amino acid transport in Schistosomes: Characterization of the permease heavy chain SPRM1hc. **The Journal of Biology Chemistry**, v. 282 (30), p. 21767-21775, 2007.

Lalla O.F., Gofman J.W. Ultracentrifugal analysis of serum lipoproteins. **Methods of Biochemical Analysis**, v.1, p.459-478, 1954.

Leal Neto, O.B., Galvão, T.Y.C., Esteves, F.A.M., Gomes, A.M.A.S., Gomes, E.C.S., Araújo, K.C.G.M., Barbosa, C.S. Análise espacial dos casos humanos de esquistossomose em uma comunidade horticultora da Zona da Mata de Pernambuco, Brasil. **Revista Brasileira de Epidemiologia**, v.15(4), p. 771-780, 2012.

Lehninger, A.L., Cox, M.M. **Princípios de Bioquímica,** 5ª ed. Porto Alegre: Artmed, 2011.

Lemmon, S.K. Clathrin uncoating:Auxilin comes to life. **Current Biology**, v.11 (2), p.49–52, 2001.

Levy-Holtzman, R., Schechter, I. Expression of different forms of the heat-shock factor during the life cycle of the parasitic helminth *Schistosoma mansoni* **Biochimica et Biophysica Acta**, v.1317, p.1-4, 1996.

Loeffler I.K., Bennett J.L., A rab-related GTP-binding protein in Schistosoma mansoni. **Molecular and Biochemical Parasitology**, v. 77, p. 31-40(10), 1996.

MacGregor, A. N., Kusel, J.R. Isolation and characterization of a surface membrane glycoprotein from adult *Schistosoma mansoni*. **Molecular and Biochemical Parasitology**, v.34, p.237-244, 1989.

Machado-Silva J.R., Galvão C., Presgrave A.O., Rey L., Gomes D.C. Host-induced morphological changes of *Schistosoma mansoni* Sambon, 1907 male worms. **Memórias do Instituto Oswaldo Cruz**, v. 89, p. 411-416, 1994.

Machado-Silva J.R., Galvão C., Oliveira R.M.F., Presgrave O.A.F., Gomes D.C. *Schistosoma mansoni* Sambon, 1907: comparative morphological studies of some Brazilian strains. **Revista do Instituto de Medicina Tropical de São Paulo**, v. 37, p. 441-443, 1995.

Machado-Silva J.R., Silva C.H., Pereira M.J.S., Pinto R.M., Oliveira R.M.F., Gomes D.C. Evaluation of differences in brazilian strains of *Schistosoma mansoni* cercariae of both sexes by means of morphometrics analysis. **Memórias do Instituto Oswaldo Cruz**, v. 95, p.839-842, 2000.

Magalhães L.A., Carvalho J.F. Estudo morfológico de *Schistosoma mansoni* pertencentes a linhagens de Belo Horizonte (MG) e de São José dos Campos (SP). **Revista de Saúde Pública de São Paulo**, v. 7, p. 289-294, 1973.

Maxfield, F.R., McGraw, T.E. Endocytic Recycling. **Nature Reviews Molecular Cell Biology,** v.5, p.121 – 132, 2004.

Mayer, M.P., Bukau, B. Hsp70 chaperones: cellular functions and molecular mechanism. **Cellular and Molecular Life Sciences**, v.62(6), p.670-668, 2005.

Meyer, F., Meyer ,H., Bueding, E., Lipid metabolism in the parasitic and free-living flat worms, *Schistosoma mansoni* and *Dugesia dorotocephala*. **Biochimica et Biophysica Acta**, v.210, p. 257-266, 1970.

Morimoto, R.I. Cells in stress: transcriptional activation of heat shock genes. **Science**, v. 259, p.1409-1410, 1993.

Mulvenna, J., Moertel, L., Jones, M.K., Nawaratna, S., Lovas, E.M., Gobert, G.N., Colgrave, M., Jones, A., Loukas, A., McManus, D.P. Exposed proteins of the *Schistosoma japonicum* tegument. **International Journal for Parasitolology**, v. 40(5), p.543-554, 2010.

Neva, F.A., Brown, H.W. **Basic Clinical Parasitology**. Appleton & Lange, p.107-144, 1994.

Neves, D.P., et al. **Parasitologia Humana**, 11ª ed. São Paulo: Atheneu, 2005.

Neves, R.H., Machado-Silva, J.R.; Pelajo-Machado, M., Oliveira, S.A., Coutinho, E.M., Lenzi, H.L., Gomes, D.C. Morphological aspects of *Schistosoma mansoni* adult worms isolated from nourished and undernourished mice: a comparative analysis by confocal laser scanning microscopy. **Memórias do Instituto Oswaldo Cruz,** v. 96 (7), p. 1013-1016, 2001.

Norman, D., Sun, X.M., Bourbon, M., Knight, B.L., Naoumova, R.P., Soutar, A.K. Characterization of a novel cellular defect in patients with phenotypic homozygous familial hypercholesterolemia. **The Journal of Clinical Investigation**, v.104, p. 619-628, 1999.

Oliveira, M.A., De Souza, W., Ferreira, S.T. Haemozoin in *Schistosoma mansoni*. **Molecular and Biochemical Parasitology**, v.111 (1), p. 217–221, 2000.

Oliveira, S.A., Barbosa Jr., A.A., Gomes, D.C., Machado-Silva, J.R., Barros, A.F., Neves, R.H., Coutinho, E.M. Morphometric Study of S*chistosoma mansoni* Adult Worms Recovered from Undernourished Infected Mice. **Memórias do Instituto Oswaldo Cruz**, v. 98(5), p. 623-627, 2003.

Pamela, C.C., Richard, A.H., Ferrier, D.R. **Bioquímica Ilustrada**. 3ed. São Paulo: Artes Médicas, 2005.

Pearse, B.M. Clathrin: a unique protein associated with intracellular transfer of membrane by coated vesicles. **Proceedings of the National Academy of Sciences**, v.73, p.1255–1259, 1976.

Pearse, B.M., Crowther, R.A. Structure and assembly of coated vesicles. **Annual Review of Biophysics and Biophysical Chemistry**, v.16, p. 49-68, 1987.

Pereira, A.S.A., Cavalcanti, N.L., Nascimento, G.A.F., Nascimento-Silva, J.L.G., Padilha, R.J.R., Viegas, L.F.W., Alves, L.C., Lima-Filho, J.L., Chaves, M.E.C. Morphological and morphometric study of cercariae and adult worms of *Schistosoma*

mansoni (SLM strain) isolated from infected mice. **Parasitology Research**, v.112, p.1087-1096, 2013.

Pernambuco, Secretaria de Saúde. Boletim de Esquistossomose e Geo helmintíases. **Vigilância em Saúde**, ano 1, n.11, dez, 2012.

Perez-Sanchez, R., Ramajo-Hernandez, A., Ramajo-Martin, V., Oleaga, A. Proteomic analysis of the tegument and excretory-secretory products of adult *Schistosoma bovis* worms. **Proteomics** v. 6(1), p. 226-236, 2006.

Pérez-Sánchez, R., Valero, M. L., Ramajo-Hernández, A., Siles-Lucas, M., Ramajo-Martín, V., Oleaga, A. A proteomic approach to the identification of tegumental proteins of male and female Schistosoma bovis worms. **Molecular and Biochemical Parasitology**, v.161, p.112-123, 2008.

Peterson, K.M., Alderete, J.F. Selective acquisition of plasma proteins by Trichomonas vaginalis and human lipoproteins as a growth requeriment for this species. **Molecular and Biochemical Parasitology,** v.12, p.37-48., 1984.

Prassl, R., Laggner, P. Molecular structure of low density lipoprotein: current status and future challenges. **European Biophysics Journal**, v.38 (2), p.145-158, 2009.

Prioli, R.P., Rosenberg, I., Shivakumar, S., Pereira, M.E.A. Specific binding of human plasma high density lipoprotein (cruzin) to *Trypanosoma cruzi*. **Molecular and Biochemical Parasitology**, v.28, p.257-264, 1988.

Rader, D.J., Hobbs, H.H. Disorders of lipoprotein metabolism. **Harrison's Principles of Internal Medicine.** 16 ed. New York, NY: McGraw-Hill Companies, Inc; Capítulo 335, p. 2287-2298, 2005.

Ramalho-Pinto, F. J., McLaren, D. J., Smithers, S. R. Complement–mediated killing of schistosomula of *Schistosoma mansoni* by rat eosinophilis *in vitro*. **The Journal of Experimental Medicine**, v. 147, p. 147-156, 1978.

Resendes, A.P.C., Souza-Santos, R., Barbosa, C.S. Internação hospitalar e mortalidade por esquistossomose mansônica no Estado de Pernambuco, Brasil, 1992/2000. **Cadernos de Saúde Pública,** v**.** 21, p. 1392-1401, 2005.

Rey, L. **Parasitologia médica** 2º ed. Rio de Janeiro: Guanabara, Cap.31-35, p.342-467, 2001.

Ribeiro P.J., Aguiar L.A.K., Toledo C.F., Barros S.M.O., Borges D.R.. Programa educativo em esquistossomose: modelo de abordagem metodológica. **Revista de Saúde Pública**, v. 38(3), p.415-421, 2004.

Rifai, N., Bachorik, P.S., Albers, J.J. Lipids, Lipoproteins and Apolipoproteins. In: Burtis CA, Ashwood E., editors. **Clinical Chemistry**. 3ed. WB Saunders & Co., 40 p. 809-861, 1999.

Rodrigues, L.E., Costa, M.F.D. Bioquímica da esquistossomose mansônica. VI - Alterações do comportamento hepático relacionado ao tempo de infecção. **Revista da Sociedade Brasileira de Medicina,** v. 20, p. 169-174, 1987.

Rogers, M.V., Henkle, K.J., Fidge, N.H, Mitchell, G.F. Identification of a multispecific lipoprotein receptor in adult *Schistosoma japonicum* by ligand blottin analyses. **Molecular Biochemistry Parasitology**, v. 35, p. 79-88, 1989.

Rogers, M.V., Quilici, D., Mitchell, G.F., Fidge, N.H. Purification of a putative lipoprotein receptor from *Schistosoma japonicum* adult worms. **Molecular and Biochemical Parasitology,** v. 41, p.93-100, 1990.

Rogers, M.V. Do Parasites express receptors for host lipoproteins? **Parasitology Today,** v. 7(5), 1991.

Ross, A. G.; Bartley, P. B.; Sleigh, A. C.; Olds, G. R.; Li, Y.; Williams, G. M.; MCManus, D. P. Schistosomiasis. **New England Journal of Medicine**, v. 346, p. 1212-1220, 2002.

Roth, M. Clathrin-mediated endocytosis before fluorescent proteins. **Nature Reviews Molecular Cell Biology**, v.7, p.63-68, 2006.

Rumjanek, F. D., McLaren, D.J., Smithers, S. R. Serum-induced expression of a surface protein in *Schistosoma mansoni:* a possible receptor for lipid uptake. **Molecular and Biochemical Parasitology**, v. 9, p. 337-350, 1983.

Rumjanek, F. D., Pereira, M. A. C., Silveira, A.M.V. The interaction of human serum with the surface membrane of Schistosomula of *Schistosoma mansoni*. **Molecular and Biochemical Parasitology**, v. 14, p. 63-73, 1985.

Rumjanek, F.D., Campos, E.G., Afonso, L.C. Evidence for the occurrence of LDL receptors in extracts of schistosomula of Schistosoma *mansoni*. **Molecular Biochemistry Parasitology**, v.28, p.145-152, 1988.

Savioli, L., Engels, D., Roungou, J.B., Fenwick, A., Endo, H. Schistosomiasis control. **Lancet**, v. 363, p. 658, 2004.

Schmid-Hempel, P. Immune defence, parasite evasion strategies and their relevance for 'macroscopic phenomena' such as virulence. **Philosophical Transactions of the Royal Socienty B**, v. 364, p. 85–98, 2009.

Schramm, G., Haas, H**.** Th2 immune response against *Schistosoma mansoni* infection. **Microbes and Infection,** v. 12, p. 881-888, 2010.

Segrest, J.P., Jones, M.K., Loof, H. Dashti, N. Structure of apolipoproteína B-100 in low density lipoprotein. **Journal of Lipid Research**, v.42(9), p.1346-1367, 2001.

Senft, A.W., Gibler, W.B. *Schistosoma mansoni* tegumental appendages: scanning microscopy following thiocarbohydrazide-osmium preparation. **The American Journal of Tropical Medicine and Hygiene**. v.26 (6), p.1169-1177, 1977.

Sher, A., Hall, B. F., Vadas, M. A. Acquisition of murine major histocompatibility complex gene products by schistosomula of *Schistosoma mansoni*. **The Journal of Experimental Medicine**, v. 148, p.46-57, 1978.

Silva, K.E.R.; Silva, R.M.F., Costa, S.P.M., Rolim, L.A.; Lima, M.C.A., Rolim-Neto, P.J. Alternativas terapêuticas no combate à Esquistossomose Mansônica. **Revista de Ciências Farmacêuticas Básica e Aplicada**, v.33(1), p. 9-16, 2012.

Smith, H. V., Kusel, J. R. The acquisition of antigens in the intercellular substance of mouse skin by schistosomula of *Schistosoma mansoni*. **Clinical and Experimental Immunology**, v. 36, p. 430-435, 1979.

Souza, D., Falcão, A.C.M.G., Gargioni, C., Kanamura, H.Y., Ciaravolo, R.M.C., Eduardo, M.B.P. Vigilância Epidemiológica e Controle da Esquistossomose: Normas e Instruções. **Centro de Vigilância Epidemiológica** "Prof. Alexandre Vranjac", Governo de São Paulo, 2007.

Stryer, L., Tymoczko, J.L., Berg, J.M. **Bioquímica.** 5ed. Rio de Janeiro: Guanabara Koogan S.A., 2004.

Tempone, A. J., Bianconi, M. L., Rumjanek, F. D. The interaction of human LDL with the tegument of adult *Schistosoma mansoni*. **Molecular and Cellular Biochemistry**, v. 177, p. 139-144, 1997.

Terlecky, S.R. Hsp70s and lysosomal proteolysis. **Experimentia**, v.50, p.1021-1025, 1994.

Torpier, C.D., Capron, A., Ouaissi, M. A. Receptor for IgG Fc ando human β-2 microglobulin on *Schistosoma mansoni* schistosomula. **Nature**, v.278, p. 447-449, 1979.

Van Balkom, B.W.M., Van Gestel, R.A., Brouwers, J.F.H.M., Krijgsveld, J., Tielens, A.G.M., Heck, A.J.R., Van Hellemond, J.J. Mass spectrometric analysis of the *Schistosoma mansoni* tegumental sub-proteome. **Journal of Proteome Research,** v.4, p. 958-966, 2005.

Van der Werf, M.J.; De Vlas, S.J.; Broker, S.; Looman, C.W.; Nagelkerke, N.J.; Habbema, J.D.; Engels, D. Quantification of clinical morbidity associated with schistosome infection in sub-Saharan Africa**. Acta Tropica**, v. 86, p. 125-139, 2003.

Van Hellemond, J.J., Retra, K., Brouwers, J.F.H.M., Van Balkom, B.W.M., Yazdanbakhsh, M., Shoemaker, C.B., Tielens, A.G.M. Functions of the tegument of schistosomes: Clues from the proteome and lipidome. **International Journal for Parasitology**, v. 36, p. 691–699, 2006.

Vitorino, R.R., Souza, F.P.C., Costa, A.P., Júnior, F.C.F., Santana, L.A., Gomes A.P. Esquistossomose mansônica: diagnóstico, tratamento, epidemiologia, profilaxia e controle. **Revista da Sociedade Brasileira de Clínica Médica de São Paulo**, v.10(1), p.39-45, 2012.

Walter, P., Lingappa, V. R. Mechanism of protein translocation across the endoplasmic reticulum membrane **Annual Review of Cell Biology**, v.2, p.499-516, 1986.

Welch, W.W., Feramisco, J.R. Nuclear and nucleolar localization of the 72,000-dalton heat shock protein in heat shocked mammalian cells. **Journal of Biological Chemistry**, v.259, p.4501-4513, 1984.

Wilson, R.A., Barnes, P.E. The tegument of *Schistosoma mansoni*: observations on the formation, structure and composition of cytoplasmatic inclusions in relation to tegument function. **Parasitology**, v. 68, p. 239-258, 1974.

World Health Organization (WHO). Schistosomiasis. n. 115, March, 2013.

Xavier, A.M., Magalhães, J.A., Cunha, G.D., Silva, A.C., Tavares, D.A., Sarro-Silva, M.D., Neto, A.H. Morphological tegument alterations of adult Schistosoma mansoni, harbored in non anti-helminthic treated, high-immune-tolerogenic and low-inflammatory mice. **Acta Tropica**, v.116, p.95-99, 2010.

Ximenes, R., Southgate, B., Smith, P., Guimarães-Neto, L. Migration and urban Schistosomiasis. The case of São Lourenço da Mata, Northeast of Brazil. **Revista do Instituto de Medicina Tropical de São Paulo**, v. 42 (4), p. 209-217, 2000.

Xu, X., Remold, H.G., Caulfield, J.P. Potencial role for scavenger receptors of human monocytes in the killing of *Schistosoma mansoni*. **American Journal of Pathology,** v.142 (3), p. 685-689, 1993.

Young, A. Structural insights into the clathrin coat. **Seminars in Cell and Developmental Biology**, v.18, p.448-458, 2007.

Zhou, M., Wu, X., Huang, L., Ginsberg, H.N. Apoprotein B100, an Inefficiently Translocated Secretory Protein, Is Bound to the Cytosolic Chaperone, Heat Shock Protein 70. **Journal of Biological Chemistry**, v.270 (2), p. 25220-25224, 1995.

4. JUSTIFICATIVA

A esquistossomose mansônica atinge cerca de 243 milhões de pessoas no mundo e mais de 6 milhões no Brasil. A morbidade e a mortalidade causadas pela doença constituem um problema de saúde pública nas regiões brasileiras afetadas, dentre as quais se inclui o estado de Pernambuco.

Alguns trabalhos sugerem que a diversidade de formas clínicas de esquistossomose parece ser devido a diferentes populações biológicas de *S. mansoni*. Assim, existe a necessidade de um estudo especifico das formas evolutivas da cepa de São Lourenço da Mata (SLM), que se acredita ser uma das grandes responsáveis pela esquistossomose mansônica humana no estado de Pernambuco.

Este é o primeiro estudo morfológico e morfométrico da cepa SLM. Com o conhecimento detalhado desta cepa pode-se compreender melhor a biologia do parasita na tentativa de esclarecer a relação parasito-hospedeiro e poder conter o avanço desta doença no estado.

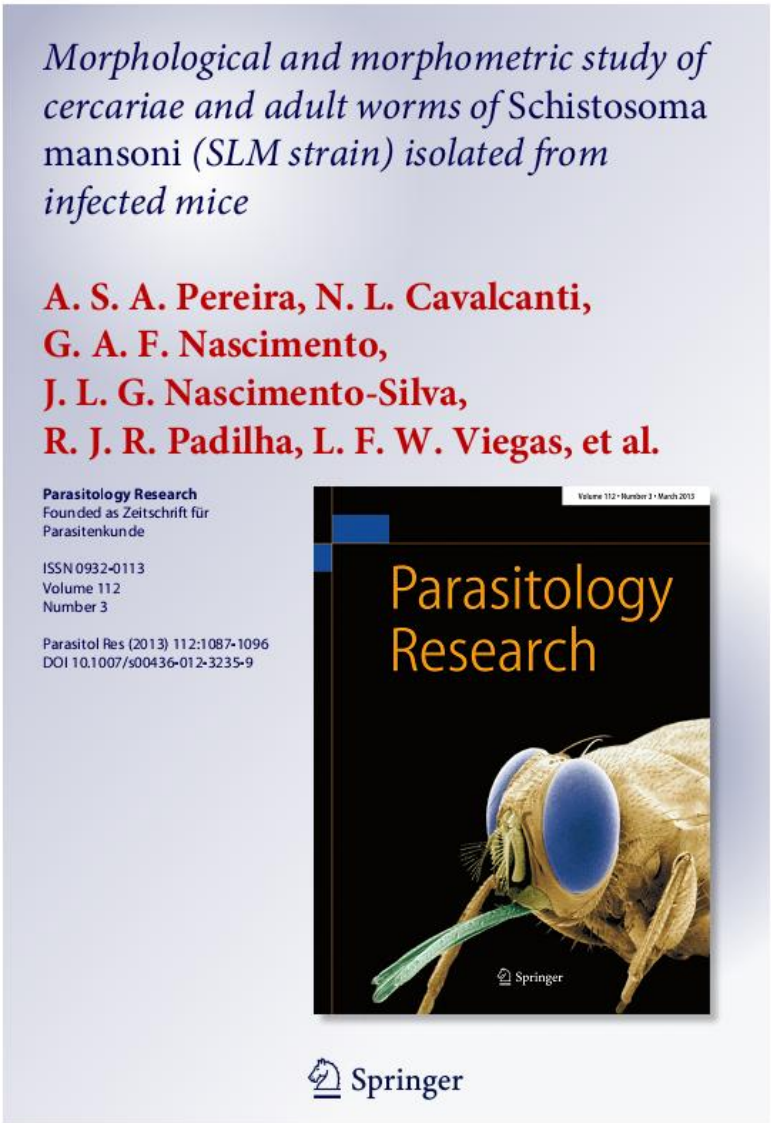
Estudos sobre esta intrigante relação acrescentam dados importantes sobre a associação com o hospedeiro e sobre as características do *S. mansoni* no Brasil, levando à conclusão de que este helminto apresenta variações morfológicas e morfometricas específicas, não podendo ser considerado como uma espécie uniforme.

Outro ponto importante no *Schistosoma mansoni* é que uma vez estabelecido em seu habitat intravascular, os vermes adquirem a capacidade de viver por muitos anos no organismo do hospedeiro, graças a várias estratégias evasivas por eles adotadas. As membranas dos parasitas se encontram em contato com o meio, estão sujeitas a inúmeras perturbações imunes, sendo estas mascaradas por moléculas do próprio hospedeiro. Como a sobrevivência do parasita depende da continua formação de novas membranas e estas necessitam de lipídeos para sua construção, é provável que estes bem adaptados parasitas adquiram os lipídeos que necessitam do próprio hospedeiro, através de mecanismos ainda não suficientemente conhecidos.

Assim, considerando a escassez desse tipo de estudo na literatura, a compreensão destes mecanismos de interação poderá ajudar a esquematizar estratégias de combate e de controle e melhorar a qualidade de vida das comunidades afetadas ou em risco de adquirir esta parasitose.

5. MORPHOLOGICAL AND MORPHOMETRIC STUDY OF CERCARIAE AND ADULT WORMS OF *SCHISTOSOMA MANSONI* (SLM STRAIN) ISOLATED FROM INFECTED MICE

Capítulo publicado na Revista: Parasitology Research



Parasitol Res (2013) 112:1087–1096
DOI 10.1007/s00436-012-3235-9

ORIGINAL PAPER

Morphological and morphometric study of cercariae and adult worms of *Schistosoma mansoni* (SLM strain) isolated from infected mice

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Received: 15 November 2012 / Accepted: 30 November 2012 / Published online: 20 December 2012

Abstract In northeastern Brazil, the schistosomiasis is historically endemic and considered as a public health problem. The *Schistosoma mansoni* São Lourenço da Mata (SLM—PE, Brazil) strain was used in several paper already published; however, morphological and morphometric studies about this strain was never done. In this work, scanning electron microscopy (SEM) was used in morphological and morphometric analysis of cercariae and adult worms. Cercariae were obtained from *Biomphalaria glabrata* snails and adult worms from mice, both infected by the *S. mansoni* SLM strain, fixed and prepared for SEM. The results showed that cercariae of *S. mansoni* measures 254.9 μm of length. The bodies are covered by spines, with a ventral sucker, an oral sucker with sensory receivers, and a pair of penetration glands in the head. The area of tail and body and the distance between suckers were 3,011.77, 1,530.32, and 42.9 μm, respectively. Adult worms of *S. mansoni* were divided into three main regions: the anterior, medial, and posterior, besides the gynecophoral canal in males. The measure of adult worms of *S. mansoni* was 4 mm males and 5 mm females. The anterior region length of the male was 470 μm and of the female 271 μm. All the parameters were assayed in ten samples. The morphometric values found in the SLM strain were smaller than other *S. mansoni* strains described in the literature as well as other helminths. This is the first morphological and morphometric study with the SLM strain of *S. mansoni* being extremely important for improving control strategies and life quality of the local population.

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Introduction

The *Schistosoma mansoni* belongs to one of three members of the genus *Schistosoma*, being the main causative agent of schistosomiasis mansonic. The schistosomiasis is one of the most important neglected tropical diseases, which affect approximately 230 million people in 77 countries and it is estimated that 747 million live under risk of infection (Engels et al. 2002; Van der Werf et al. 2003; Gryseels et al. 2006; Attallah et al. 2008; WHO 2012).

Schistosomiasis in Brazil remains an important disease in the context of public health with an estimated number of 2.5 million people and 26 million at risk of infection (Brazil 2005; Vitorino et al. 2012). In northeastern Brazil, schistosomiasis has been widely distributed. The endemic areas are located preferentially in communities with precarious conditions of hygiene and inadequate sanitary resources (Barbosa and Barbosa 1998; Barbosa et al. 2001; Rouquayrol and Filho 2003). The state of Pernambuco presents this helminthiasis endemic in 102 of the 186 municipalities of the state, and is considered one of the states with the highest prevalence, especially in forest areas, coastline, and in cities of the edges of Capibaribe river (Katz and Peixoto 2000; FUNASA 2002; Brasil 2011). The city of São Lourenço da Mata is localized in

forest area, has a hot and humid climate, where the average annual temperature is 27 °C, with small variations between the dry and rainy seasons (Barbosa and Silva 1992).

Environmental factors favored the spread of disease in tropical areas, due to high temperatures, humidity, and presence of the intermediate host (snails of the genus *Biomphalaria glabrata* and *Biomphalaria straminea*) (Souza et al. 2011). In the life cycle of *Schistosoma*, the intermediate host plays a key role in maintenance of parasitosis (Schneider and Zelck 2001). The ultrastructural changes in the miracidium body of *S. mansoni* occur within 48 h after penetration in the intermediate host (Meuleman et al. 1978). The infection in the definitive host, human or animal, occurs through of cercariae penetration in the skin, using the oral sucker and associated duct, thus reaching the bloodstream. In the vascular system, the schistosomula mature, becoming in adult worms, males and females copulate and lay their eggs in feces (Robson and Erasmus 1970; Van Hellemond et al. 2006).

The cercariae, one of the *S. mansoni* evolutive forms, are specialized in performing the functions of locomotion, invasion host, and maturation in adult worms. It measures around 500 μm, which can vary considerably due to its ability to contraction and longitudinal elongation. The ability of cercariae to stretch depends on the size of the basal lamina fibrous, it presents a high volume of muscle cells needed to vigorous movement during swimming, contraction, and extension of the tail, body, and suckers (Dorsey et al. 2002; Cavalcanti et al. 2009).

The adult worms have three main areas of analysis: anterior, medial, and posterior. The anterior region of adult worms of both sexes is presented in a similar way with oral and ventral suckers. The tegument median of male adult worm proves more complex than the female. Tubercles are distributed over the entire extension of the medial region in the male parasite; its surface is very wrinkled and grooved and exist a significant presence of spines (Hockley 1973; Lopez-Alvarez 1980; Pereira et al. 2011). The ventral area of dorsal region have formation of gynecophoral canal, where it is kept by the female during mating, their borders and interior are covered with spines suggesting that these structures are related to the mating of the worms (Miller et al. 1972; Machado-Silva et al. 1997; Pereira et al. 2011).

The tegument of schistosomes is a dynamic host-interactive layer involved in nutrition, immune evasion and modulation, excretion, osmoregulation, sensory reception, and signal transduction (Jones et al. 2004; Van Hellemond et al. 2006). Adult schistosomes are long-lived and are able to avoid immune-mediated clearance from the host (Kusel et al. 2007). The ability of schistosomes to avoid host immune responses can be attributed in part to the dynamic nature of their host-exposed outer covering, or tegument, and the complex immuno-evasive strategies they employ to avoid elimination from the harsh intravascular environment (Pearce and MacDonald 2002; Abdul-Ghani 2009).

Morphological and morphometric studies have been proposed to explain taxonomic questions to several species of different strains of *S. mansoni* that infect humans (Machado-Silva et al. 1994; Oliveira et al. 2003). With the technological evolution of structural analysis equipment, a detailed study of parasites regions not previously explored could be made. Several authors employ morphometry of adult worms and cercariae to characterize strains of *S. mansoni* (Magalhães and Carvalho 1973; Machado-Silva et al. 1995, 2000; Neves et al. 2002). The morphological study of adult worms and cercariae can appear different depending on the strain or species. This work describes the morphology of the evolutionary forms (adult worm/cercariae) of *S. mansoni* São Lourenço da Mata (SLM) strain through scanning electron microscopy (SEM). A structural characterization was never done in order to evidence possible features changes over time. These data can be used to better understand the biology of the parasite that affects our region and to clarify the intriguing host–parasite relationship.

Materials and methods

Maintenance of parasite life-cycle

Eggs of *S. mansoni* were collected from excrements originating from individuals native of the city of São Lourenço da Mata, Pernambuco, Brazil, after reading and signing the terms of agreement. Parasitological analyses were done through the method of Kato-Katz (Katz et al. 1972). The *B. glabrata* snails were placed individually in wells of culture plates containing 3 mL of distilled water, where were added eight to ten miracidia of *S. mansoni* per well. A total of 68 snails were infected, for a period of 2 h, under heat and intense light.

After 30 days of infection, the snails were displayed to the heat and the intense light for 2 h, for elimination of the cercariae that were used in the morphometric analysis and for the male mice (*Mus musculus*) infection. The evolutive cycle of the parasite was kept in the Laboratory of Imunopatology Keizo Asami (LIKA), Federal University of Pernambuco.

Mice with 7–9 weeks of age, weighing 35–45 g were housed in cages (30×20×13 cm) containing sterile wood shaving bed. Standard diet (Labina®, Ralston Purina Ltda, São Paulo, Brazil) and water were available ad libitum. Room temperature was kept at 22±2 °C and 12:12 h light/dark cycle. Mice were infected individually with 100 *S. mansoni* cercariae, under artificial light for at least 2 h. This study was approved by the ethical committees for research with animals of the Federal University of Pernambuco, Brazil.

Scanning electron microscopy of *S. mansoni*

Sixty days post-infection, animals were euthanized and adult worms collected by the perfusion of the hepatic portal system with saline solution (0.85 % of NaCl, p/v) (Duvall and Dewitt 1967). The worms and cercariae were washed several times with phosphate-buffered saline, pH 7.4, and fixed for SEM. Cercariae and adult worms were fixed in AFA (alcohol 85 %, formaldehyde 10 %, and acetic acid PA 5 %) at 4 °C. After the fix stage, the material was washed with sodium cacodylate buffer (0.1 mol/L, pH 7.2) and post-fixed with 1 % (*w*/*v*) osmium tetroxide for 1 h. They were dehydrated through a graded series of ethanol, dried in a Hitachi HCP-2 critical point dryer machine using liquid carbon dioxide as a transitional medium. After drying, they were mounted on aluminum stubs and coated with gold in an ion-sputtering apparatus, FINE-COAT 1100-JEOL (Ion Sputter JFC-1100; Tokyo, Japan), at 1.2 kV, 10 mA for 6 min (Storey and Ogbogu 1991).

Morphometric analysis

The samples were examined and photographed in a scanning electron microscopy JEOL-JSM-5600 LV (Tokyo, Japan), operating at 10 kV. Different areas and structures of body of both cercariae and adult worms of *S. mansoni* were analyzed. Measures were made in a computerized image analyzer system (Image-Pro Plus, Media Cybernetics).

Results

Studies showed that cercariae of *S. mansoni* are divided into oral region (head), body, and caudal region (Fig. 1 (1)). In our study the average length of cercariae of *S. mansoni* (n=10) was 254.9 μm since its caudal extremity until oral extremity varying of 174 to 290 μm of total length. The size of the oral region was 23.7 μm, while the body and the tail were 59.3 and 171.9 μm, respectively (Fig. 1 (2)).

The cercariae body of *S. mansoni* presented coated spines, where the number and salience of spines projections are less prominent in the body than the tail, showing the difference in the composition of the tegument. Beyond the coated spines, the presence of sensorial cilia and a connection region between the body and tail was observed (Fig. 1 (3)). The average distance between oral suckers until the connection region was 82.4 μm (Fig. 1 (4)). It was also investigated the area measure of some characters of SLM strain (Table 1).

The presence of a sensory receptors necklace composed for cilia measuring approximately 2.18 μm was seen in the region between the head and the body of *S. mansoni* cercariae (Fig. 1 (5)). Our studies of the oral region showed the presence of a pair of sensory glands which could be retracted for the interior of sucker or projected for its exterior (Figs. 1 (6) and 2 (7)). The diameter average of the *S. mansoni* cercariae oral sucker was 11.3 μm.

The *S. mansoni* cercariae tail surface showed spines organized in a parallel form with the pointed extremity distributed in all its extension (Fig. 2 (8)). This structure is related to the locomotion of the larva in the water. Ciliary projections with sensory function were identified randomly distributed in the tail, beyond the presence of a bifurcation in its final portion. The presence of an excretory pore at the extremity of the bifurcate tail was also observed, with opening 1.42 μm without the presence of spines in this region (Fig. 2 (9 and 10)).

Adult worms of *S. mansoni* (n=10) were divided into three main regions: the anterior, medial, and posterior. The gynecophoral canal is present only in males (Fig. 3 (11 and 12)). The measure of adult worms of *S. mansoni* males was about 4 mm (Fig. 3 (13)) and females 5 mm (Fig. 3 (14)).

The anterior region consists in the head of the parasite. Male and female anterior region showed a tegument smooth and porous, containing small spines and the oral and ventral suckers (Fig. 3 (15 and 16)). In the suckers were found a layer of small spines that can act as sensory receptors of the parasite.

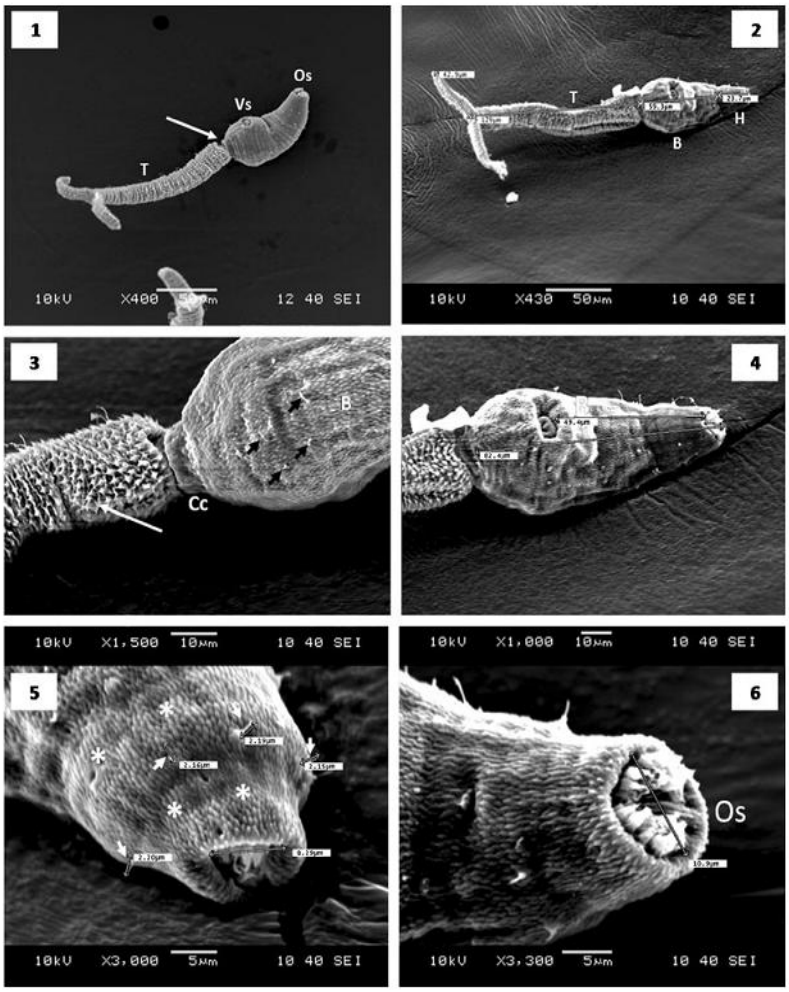
The diameters of the ventral suckers of male and female were 197 and 40 μm, respectively. The ventral suckers edges were 23 μm in the male and 7 μm in the female (Fig. 3 (15 and 16)).

There was a considerable difference between the parameters obtained in the anterior region of male and female worms (Table 2).

The male oral and ventral suckers differed in their architecture. The edge of ventral sucker was completely covered by spines with the inside showing small folds and a smoother tegument, while the oral sucker presents completely covered with spines (Fig. 4 (17 and 18)).

The tegument medial region of the *S. mansoni* male showed a large number of tubercles uniformly distributed between the folds measuring approximately 34.4 μm in length and 12.6 μm in width (Fig. 4 (19)). A large number of spines could be seen covering the tubercles, however, they did not appear between the channels and grooves that make up the wrinkled area of the surface (Fig. 4 (19)). The width of the dorsal tegument of *S. mansoni* male adult worms was 1.15 mm, this portion showed many pores in the folds, which may be involved in the absorption of nutrients by the parasite (Fig. 4 (20)). The medial region of the female tegument became more delicate, without the presence of spines or tubercles, but also presented pores and sensory papillae (Fig. 4 (21 and 22)).

The gynecophoral canal shelter the female for reproduction (Fig. 5 (23 and 24)). Spines emerging from many pores forming a uniform layer that takes the entire region inside



Figs. 1–6 Micrographs of the *S. mansoni* cercariae. *1* Cercariae showing the three main regions (*bar*=500 µm). *2* Cercariae showing the three main regions with measures (*bar*=50 µm). *3* Connection region between the body and tail cercariae (*bar*=10 µm). *4* Measures of anterior region and distance between suckers (*bar*=10 µm). *5* Presence of sensory receptors composed for cilia (*bar*=5 µm). *6* Oral sucker presenting sensorial glands (*bar*=5 µm)

the channel (Fig. 5 (25)). It has been proposed that the area of male–female contact can serve to transfer amino acids and other small molecules easy diffusion (Senft and Gibler 1977).

When approaching the posterior region of the male parasite, the tegument becomes more wrinkled than in other regions (Fig. 5 (26)). Some bulges between the grooves were still observed, suggesting the beginning of the growth of new tubercles. Differently of other tegument regions, the distal extremity of male, that has an average length of 270 µm, did not present spines or tubercles. In this area was observed an excretory pore surrounded by a porous region (Fig. 5 (27)). The posterior region of the female tegument, with average length of 110 µm, appeared smooth with its final extremity recovered with spines and presented an excretory pore as seen in the male (Fig. 5 (28)).

Table 1 Morphometric data of characters in SLM strain of *S. mansoni* cercariae

Characters	Cercariae (mean ± SD)
Body area (µm^2)	1,530.32±115.61
Oral sucker area (µm^2)	92.5±23.34
Ventral sucker area (µm^2)	82.52±18.98
Tail area (µm^2)	3,011.77±414.86
Distance between suckers (µm)	42.9±4.66

Discussion

The morphology of trematodes was studied by several research groups since 1857 until today, but there are few reports on morphometric analysis aiming taxonomic approaches (Coles and Thurston 1970; Magalhães and

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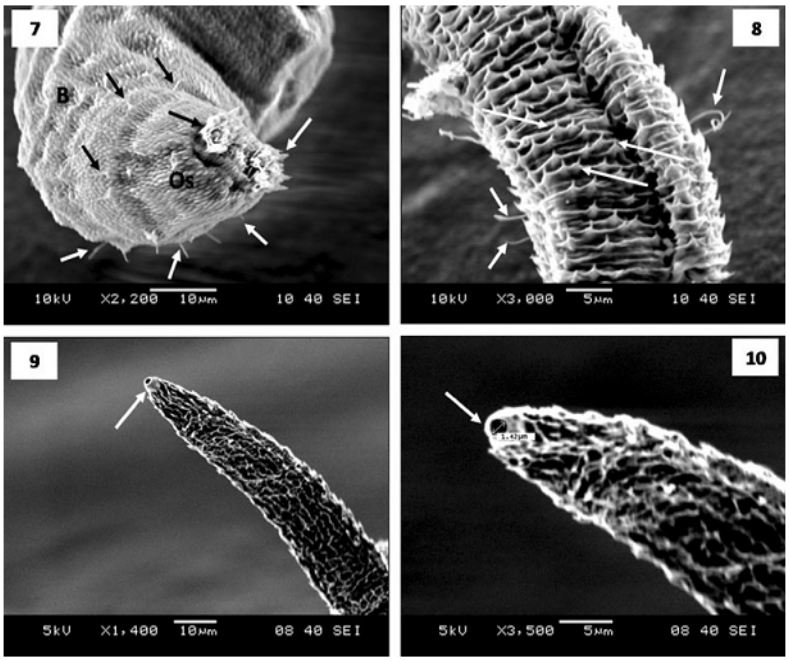


Figs. 7–10 Scanning electron micrographs of the *S. mansoni* cercariae. *7* Oral sucker with a pair of sensorial glands (*bar*= 10 µm). *8* Surface cercariae tail re-covered with spines and sensorial cilia (*bar*=5 µm). *9* Excretory pore at the bifurcate tail (*bar*=10 µm). *10* Excretory pore at the bifurcate tail with diameter measure (*bar*=5 µm)

Carvalho 1973; Paraense and Côrrea 1981; Machado-Silva et al. 1994, 1995; Neves et al. 1998; Dorsey et al. 2002; Cavalcanti et al. 2009). In the present study was used scanning electron microscopy to characterize the ultrastructure of cercaria and adult worms of *S. mansoni* isolated from patients living in the city of São Lourenço da Mata, Pernambuco, Brazil.

Data related on the morphological division of cercariae found in our study also was proposed by authors working with cercariae *Echinostoma caproni*, *Allassonoporus* sp., and *Allopodocotyle* sp. (Bogéa and Caira 2001a, b; Nakano et al. 2003). In accordance with the size, the *Cercaria misenensis* (marine cercaria) presents measure of body and tail about 119 to 97 µm, respectively, totaling 216 µm full length (Dolgikh 1965), while cercariae *Austrobilharzia* sp were described with body dimensions 328 µm long (Abdul-Salam and Sreelatha 2004). Similar measures were reported to *Cercaria sevillana* being the size of body 122 and 106 µm the tail (Russel-Pinto and Bartoli 2002).

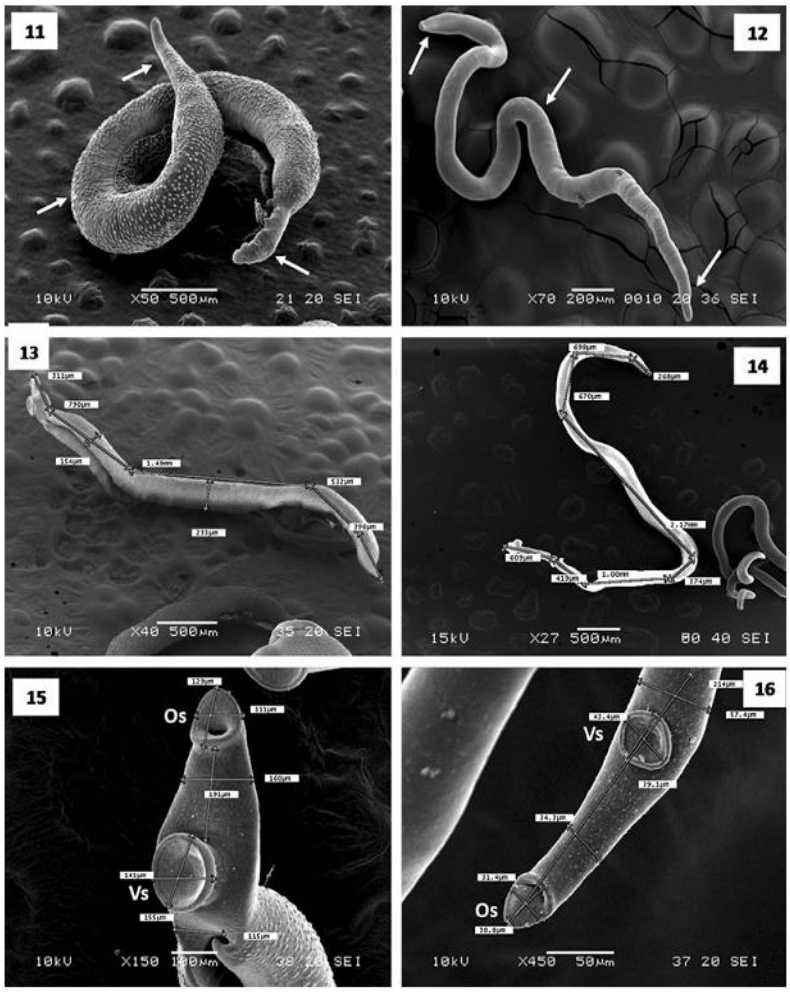
The average measure of various strains of *S. mansoni* cercariae may achieve up 500 µm and this size varies according to the ability of contraction and elongation (Dorsey et al. 2002). Results of this work (maximum length=290 µm) differ from reported previously to cercariae of *S. mansoni*, approaching to the values found by the authors mentioned above, in studies with cercariae of different species. Our observation increases literature data with the measure of average thickness of the larvae to the three regions (tail, body, and head) which were 18, 33, and 20 µm, respectively. It was observed the presence of a connection region between the body and tail of the cercaria, called the body–tail junction that corroborate with other authors (Dorsey et al. 2002).

The suckers showed in the larva have important role in the process of penetration in the definitive host. They are fixed between the hair follicles making possible that the invasion occurs in an active form by the alternating contraction and relaxation of the oral sucker and body musculature assisted by histolytic secretions from pairs of penetration gland cells liberated during the incoming larva (Robson and Erasmus 1970; Rey 2002; Souza et al. 2011).

The measure of the oral sucker opening of the *S. mansoni* larva, SLM strain was constant (11.3 µm) in all analyzed sample, although differences have been found in the same strain of *C. misenensis* larva (20 and 30 µm) by other authors (Palombi 1940; Dolgikh 1965).

The *S. mansoni* cercariae SLM strain, showed a pair of sensorial glands in the oral sucker. Similar sensorial glands were observed in cercariae of the genus *Trichobilharzia* and also in *Allopodocotyle* sp. (Kock and Böckeler 1998; Bogéa and Caira 2001b). The genus *Trichobilharzia* presents four types of sensory cilia, it is believed that the presence of sensorial receptors on cercaria is related to the larvae–host invasion (Kock and Böckeler 1998; Russel-Pinto and Bartoli 2002).

The maturation rate of cercariae to adult worms, which leads to the infectivity level, is also a criterion for the differentiation of strains. In our study the values obtained (30 %) are among the standards for Brazilian strains from 21 to 61 % (Anderson and Cheever 1972).



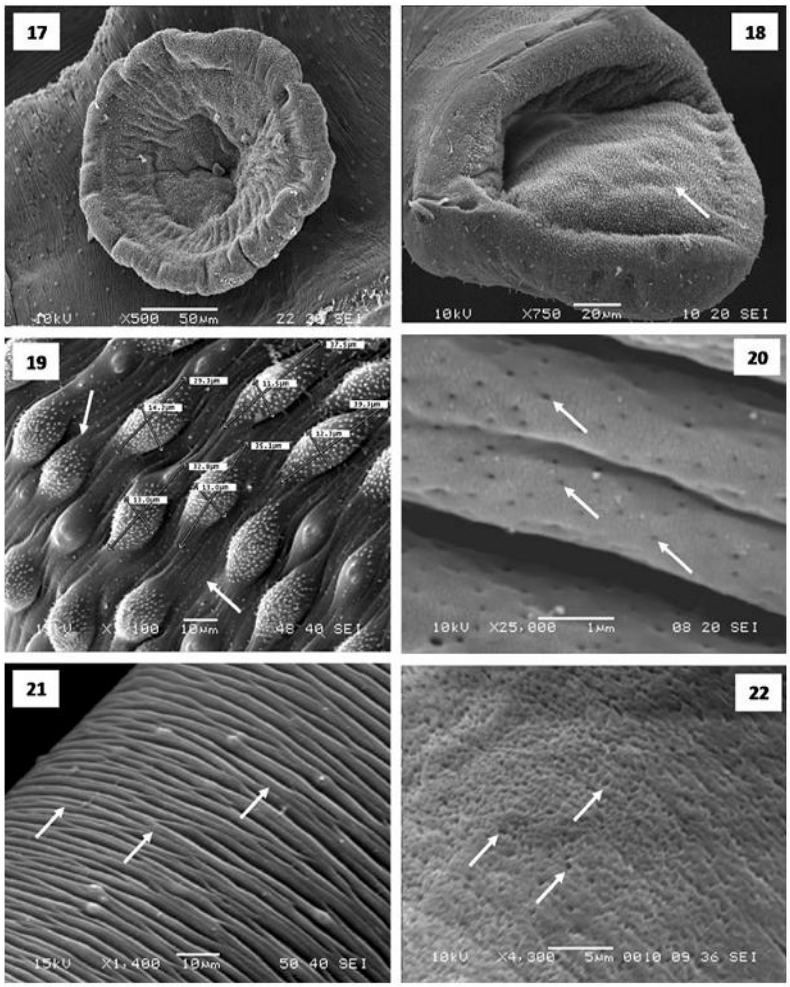
Figs. 11–16 Micrographs of the adult worms of *S. mansoni*. *11* Male worm showing the three main regions (*bar*= 500 µm). *12* Female worm showing the three main regions (*bar*=200 µm). *13* Measures of length body of male parasite (*bar*=500 µm). *14* Measures of length body of female parasite (*bar*=500 µm). *15* Anterior region of male worm presenting suckers oral and ventral (*bar*=100 µm). *16* Anterior region of female worm presenting suckers oral and ventral (*bar*=50 µm)

It was verified that Brazilian strains of *S. mansoni* have showed morphometric differences among themselves. The literature data indicate that morphometric values of the oral suckers, the ventral suckers, and the tail areas were divergent between SJ (São José dos Campos, São Paulo, Brazil) strain 310, 200, and 3,900 µm^2, BH (Belo Horizonte, Minas Gerais, Brazil) strain 610, 380, and 7,400 µm^2, and CMO (Ceará-Mirim, Rio Grande do Norte, Brazil) strain 490, 300, and 4,960 µm^2, respectively (Machado-Silva et al. 2000). In the present study, the oral suckers measured 92.5 µm^2, the ventral suckers 82.52 µm^2, and the tail areas 3,011.77 µm^2. The distance between the oral and the ventral suckers were 40 µm (SJ and CMO strains), 60 µm (BH strain) and 49 µm (SLM strain). Comparing our results with the different strains of *S. mansoni* above referred, it could be observed that the values of oral suckers, ventral suckers, and tail areas were smaller than the other strains, while the distance between suckers was closest to SJ and CMO strains.

Table 2 Morphometric data of the anterior region in SLM strain of *S. mansoni* adult worms

Characters	Male (mean ± SD)	Female (mean ± SD)
Head (µm)	422.52±68.88	198.93±37.4
Distance between suckers (µm)	162.69±21.66	90.32±16.5
Oral sucker area (µm^2)	6,138.88±1,482.47	753.36±170.01
Ventral sucker area (µm^2)	23,021.28±7,301.09	1,063.87±253.72

The uniform distribution of spines along the full extent of tail tegument, the presence of sensorial cilium, and excretory pore visualization at the final extremity of tail bifurcation, coincide with results of cercariae from *Trichobilharzia*



Figs. 17–22 Scanning electron micrographs of suckers and dorsal medial region of the adult worm of *S. mansoni*. *17* Ventral sucker of male worm (*bar*=50 μm). *18* Oral sucker of male worm (*bar*=20 μm). *19* Tubercles in the median region of male worm (*bar*= 10 μm). *20* Possible absorption pore in medial region of adult male worm (*bar*=1 μm). *21* Medial region of female parasite presented grooves and papilla (*bar*=10 μm). *22* Possible absorption pores in medial region of female worm (*bar*=5 μm)

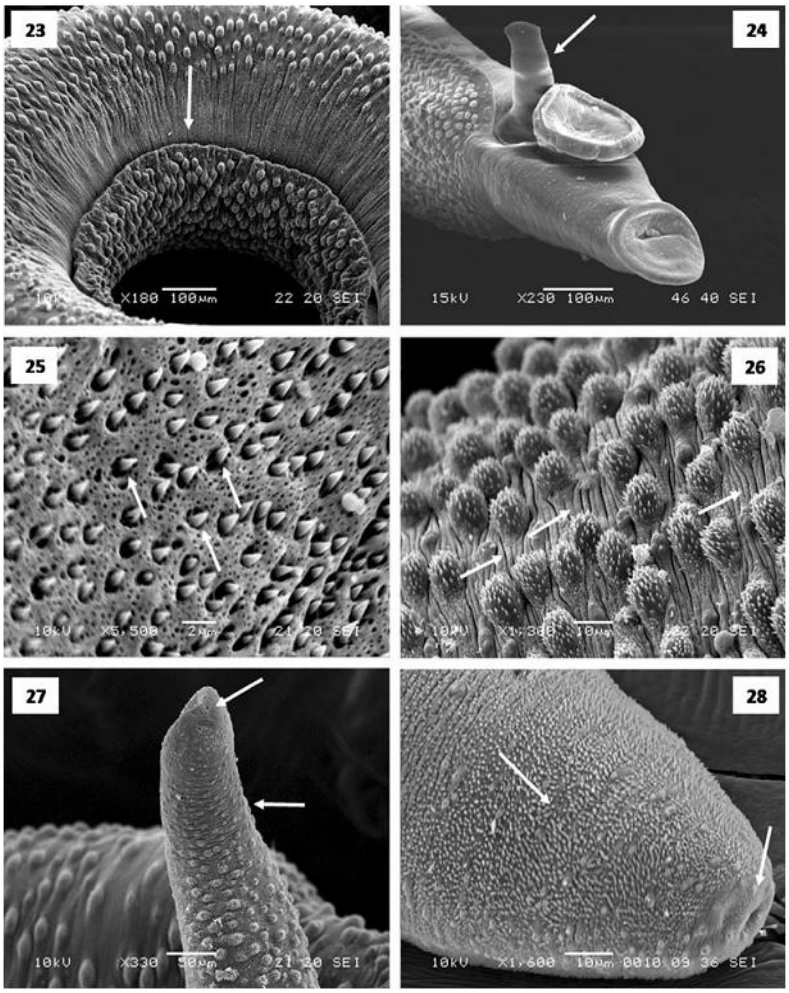
ocellata (Kock and Böckeler 1998). In contrast, in *E. caproni* the tail was coated by membrane with wavy projections, besides this, it was found the presence of ciliary processes and the absent of fork in its extremity (Nakano et al. 2003). Peaked spines were described uniformly distributed throughout the body of *C. sevillana* (Russell-Pinto and Bartoli 2002). *Schistosoma margrebowiei* cercariae presents spines more concentrated on the head than on body and tail (Southgate and Knowles 1977). Differently from the Nakano (2003) and Russell-Pinto and Bartoli (2002) studies, in this work the number and bulges of the spines projections were more prominent on the tail than on the body.

The *S. mansoni* adult worms exhibit significant morphological (Sobhon et al. 1986) and morphometric differences according to their species (Hockley 1973; Kuntz et al. 1976; Hicks and Newmann 1977; Oliveira et al. 2003; Neves et al. 2005).

According to the length of the adult worms, *S. mansoni* Egyptian strains (Beni-Suef and Giza) (Soliman et al. 1986) were 33–45 % higher than the SLM strain. Comparing the *S. mansoni* SLM strain with other Brazilians strains, it has more similarities to the SJ than BH, CMO, and SNN (Sumidouro, Rio de Janeiro, Brazil). The three last strains are 30–50 % higher than SLM (Machado-Silva et al. 1995; Neves et al. 1998; Machado-Silva et al. 2003).

The area values of the oral and ventral suckers found in the literature were only referred to the SNN strain, which measured 21,959 and 28,716 μm^2 in male and 2,478 and 2,739 μm^2 in female, respectively (Neves et al. 1998). In our work, the SLM strain showed to be smaller than SNN with oral and ventral suckers measuring 6,138.88 and 23,021.28 μm^2 in male and 753.36 and 1,063.87 μm^2 in female, respectively.

Comparing the distance between oral and the ventral suckers in male and female found in this work (162.69 and



Figs. 23–28 Micrographs of the posterior region and gynecophoral canal of adult worms of *S. mansoni*. *23* Gynecophoral canal of male adult worm (*bar*=100 μm). *24* Female worm inside in gynecophoral canal (*bar*=100 μm). *25* Surface of gynecophoral canal (*bar*=2 μm). *26* Tubercles in the posterior region of male worm (*bar*=10 μm). *27* Excretory orifice of male parasite (*bar*=50 μm). *28* Excretory orifice of female adult worm (*bar*=10 μm)

90.32 μm, respectively) with SNN strain (283 and 212 μm) (Neves et al. 1998) they differed from ours results. Concern to male worms, the values found to BH (230 μm), CMO (180 μm), and SJ (220 μm) strains were higher than SLM (Martinez et al. 2003; Oliveira et al. 2003). Two areas free of spines were found within the male ventral sucker of *S. mansoni* SLM strain, similar results were also in *Schistosoma japonicum* worms (Sobhon et al. 1986).

Topographical characteristic have been related in the anterior region of the oral and the ventral suckers. In those region the *S. japonicum* and *Schistosomatium douthitti* tegument is less folded than other regions and is composed of grooves and channels (Gupta and Basch 1988; Gobert et al. 2003), differently of the porous aspect containing small spines showed in our results and in other species of *Schistosoma* such as *Schistosoma haematobium*, *Schistosoma mattheei*, and *Schistosoma mekongi* (Sobhon et al. 1986; Gupta and Basch 1988; Jiraungkoorskul et al. 2006).

The surfaces of the dorsal and posterior regions of several species of male *Schistosoma* are characterized by the presence of numerous tubercles with a high concentration of spines (Sobhon et al. 1986; Gupta and Basch 1988; Jiraungkoorskul et al. 2006), the same aspects were observed in this work. Previous work with the *S. haematobium* has showed that the surface of the male worm is similar to SLM strain, except for the presence of an increased number of tubercles in the dorsal region and a larger amount of spines. In the *S. mansoni* female it was observed a smooth tegument without the presence of tubercles, but with small spines and papillae distributed over its surface, whereas in *S. haematobium* small protrusions without spines were present (Kuntz et al. 1976; Hicks 1977; Burden and Ubelaker 1981).

The area between the tubercles of *S. mekongi* (Jiraungkoorskul et al. 2006), *S. haematobium* (Burden and Ubelaker 1981), *S. mattheei* (Gupta and Basch 1988) and *Schistosoma leiperi* (Southgate et al. 1981) was composed of folds, bulbous with cilia, small pores, and diminutives spines that were also seen in the SLM strain. The presence of a cilium on the bulbous indicates that these papillae may be tactile receptors (Hockley 1973).

The teguments of *S. japonicum* (Chinese, Philippine, and Indonesian strains) and *S. douthitti* male worm are highly folded resulting in a sponge-like appearance and in females they are formed by smooth surfaces with low parallel ridges (Sobhon et al. 1986; Gupta and Basch 1988; Gobert et al. 2003). In the *S. mansoni* SLM strain no sponge-like appearance was observed on the tegument of male worms, however it displayed grooves around the tubercles. Females of the SLM strain did not present parallel ridges as was seen in other species above referred.

Confirming anterior data (Sobhon et al. 1986; Gupta and Basch 1988; Gobert et al. 2003; Pereira et al. 2011), male and female of *S. mansoni* possessed an excretory pore in posterior region.

This work has provided important information about morphology and morphometry of various structures that comprise the *S. mansoni* SLM strain, as well as related structures with various functions performed by evolutive forms, since cercariae until adult worm. It is believed that SLM strain is the major responsible for the human schistosomiasis mansoni in the state of Pernambuco, Brazil, being extremely important a detailed knowledge of this strain for improving control strategies and life quality of the local population.

Acknowledgments This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). The authors would like to thank Dr. Humberto Bertão for their technical assistance.

References

Abdul-Ghani R, Loutfy N, El Sahn A, Hassan A (2009) Current chemotherapy arsenal for *Schistosomiasis mansoni*: alternatives and challenges. Parasitol Res 104:955–965

Abdul-Salam JB, Sreelatha S (2004) Description and surface topography of the cercaria of *Austrobilharzia* sp. (Digenea: Schistosomatidae). Parasitol Intern 53:11–21

Anderson LA, Cheever AW (1972) Comparison of geographical strains of *Schistosoma mansoni* in the mouse. Bull World Health Organ 46:233–242

Attallah AM, Wahba MA, Elsheikha HM, Abbas AT, Abdel Aziz MM, El-Hemaly MA (2008) Outcomes of *Schistosoma mansoni* infection in outbred albino mice exposed to Larvin contaminant. Parasitol Res 103:567–576

Barbosa CS, Silva CB (1992) Epidemiologia da Esquistossomose mansônica no Engenho Bela Rosa, Município de São Lourenço da Mata, Pernambuco. Brasil Cad Saúde Públ 8(1):83–87

Barbosa CS, Barbosa FS (1998) Padrão epidemiológico da esquistossomose em comunidade de pequenos produtores rurais de Pernambuco. Brasil Cad Saúde Públ 14(1):129–137

Barbosa CS, Domingues ALC, Abath A (2001) Epidemia de esquistossomose aguda na praia de Porto de Galinhas. Pernambuco Brasil Cad Saúde Públ 17(3):725–728

Bogéa T, Caira JN (2001a) Chaetotaxy and ultrastructure of sensory receptors in the cercaria of a species of *Allassogonoporus* Olivier, 1938 (Digenea: Lecithodendriidae). Syst Parasitol 50:1–11

Bogéa T, Caira JN (2001b) Ultrastructure and chaetotaxy of sensory receptors in the cercaria of a species of *Allopodocotyle* Pritchard, 1966 (Digenea: Opecoelidae). Mem Inst Oswaldo Cruz 96(2):205–214

Brasil (2005) Ministério da Saúde. Secretaria de Vigilância em Saúde. Guia de vigilância epidemiológica, 6rd edn Brasília, Brasil, pp 297–298

Brasil (2011) Ministério da Saúde. Secretaria de Vigilância em Saúde. Sistema Nacional de Vigilância em Saúde, Relatório de Situação, Pernambuco, 5rd edn Brasília, Brasil, p.7

Burden CS, Ubelaker JE (1981) *Schistosoma mansoni* and *Schistosoma haematobium*: differences in development. Exp Parasitol 51:28–34

Cavalcanti MGS, Araújo HRC, Paiva MHS, Silva GM, Barbosa CCGS, Silva LF, Brayner FA, Alves LC (2009) Ultrastructural and cytochemical aspects of *Schistosoma mansoni* cercaria. Micron 40:394–400

Coles GC, Thurston JP (1970) Testes number in East African *Schistosoma mansoni*. J Helminthol 44:69–73

Dolgikh, AV (1965) Trematode larvae, parasites in the Black Sea mollusk *Nassa reticulata* var. pontica Mont. Biologiya Morya Bentos Kiev. Nauk Dumka 122–138

Dorsey CH, Cousin CE, Lewis FA, Stirewalt MA (2002) Ultrastructure of the *Schistosoma mansoni* cercaria. Micron 33(3):279–323

Duvall RH, Dewitt WB (1967) An improved perfusion technique for recovering adults schistosomes from laboratory animals. AmJ-Trop Med Hyg 16:483

Engels D, Chitsulo L, Montresor A, Savioli L (2002) The global epidemiological situation of schistosomiasis and new approaches to control and research. Acta Trop 82:139–146

FUNASA (2002) Fundação Nacional de Saúde Guia de Vigilância Epidemiológica, 1

Gobert GN, Stenzel DJ, McManus DP, Jones MK (2003) The ultrastructural architecture of the adult *Schistosoma japonicum* tegument. Int J Parasitol 33:1561–1575

Gryseels B, Polman K, Clerinx J, Kestens L (2006) Human schistosomiasis. Lancet 368:1106–1118

Gupta BC, Basch PF (1988) Surface maturation in female *Schistosoma mansoni*, *S. mattheei* and *Schistosomatium douthitti*. Int J Parasitol 18(2):275–280

Hicks RM, Newmann J (1977) The surface structure of the tegument of *Schistosoma haematobium*. Cell Biol Intern Rep 1:157–167

Hockley DJ (1973) Ultrastructure of tegument of Schistosoma. Adv Parasitol 11:233–305

Jiraungkoorskul W, Sahaphong S, Sobhon P, Riengrojpitak S, Kangwanrangsan N (2006) *Schistosoma mekongi*: the in vitro effect of praziquantel and artesunate on the adult fluke. Exp Parasitol 113:16–23

Jones MK, Gobert GN, Zhang L, Sunderland P, McManus DP (2004) The cytoskeleton and motor proteins of human schistosomes and their roles in surface maintenance and host–parasite interactions. BioEssays 26:752–765

Katz N, Chaves A, Pellegrino JÁ (1972) Simple device for quantitative stool thick-smear technique in mansonic schistosomiasis. Rev Inst Med Trop São Paulo 14:397–400

Katz N, Peixoto SV (2000) Análise crítica da estimativa do número de portadores de esquistossomose mansônica no Brasil. Rev Soc Bras Med Trop 33:3

Kock S, Böckeler W (1998) Observations on cercarial chaetotaxy as a means for the identification of European species of *Trichobilharzia* Skrjabin & Zakharow, 1920 (Digenea: Schistosomatidae). Syst Parasitol 43:159–166

Kuntz RE, Tulloch GS, Davidson DL (1976) Scanning electron microscopy of the integumental surfaces of *Schistosoma haematobium*. J Parasitol 62:63–69

Kusel JR, Al-Adham BH, Doenhoff MJ (2007) The schistosome in the mammalian host: understanding the mechanisms of adaptation. Parasitol 134:1477–1526

López-Alvarez ML (1980) Contribuição ao estudo ultra-estrutural do *Schistosoma mansoni* Sambon,1907. Dissertation, Federal University of Rio de Janeiro

Machado-Silva JR, Galvão C, Presgrave AO, Rey L, Gomes DC (1994) Host-induced morphological changes of *Schistosoma mansoni* Sambon, 1907 male worms. Mem Inst Oswaldo Cruz 89:411–416

Machado-Silva JR, Galvão C, Oliveira RMF, Presgrave OAF, Gomes DC (1995) *Schistosoma mansoni* Sambon, 1907: comparative morphological studies of some Brazilian strains. Rev Inst Med Trop São Paulo 37:441–443

Machado-Silva JR, Lanfredi RM, Gomes DC (1997) Morphological study of adult male worms of *Schistosoma mansoni* Sambon: 1907 by scanning electron microscopy. Mem Inst Oswaldo Cruz 92(5):647–653

Machado-Silva JR, Silva CH, Pereira MJS, Pinto RM, Oliveira RMF, Gomes DC (2000) Evaluation of differences in Brazilian strains of *Schistosoma mansoni* cercariae of both sexes by means of morphometrics analysis. Mem Inst Oswaldo Cruz 95:839–842

Machado-Silva JR, Neves RH, Ormond L, Hulstijn M, Gomes DC (2003) Caracterização de cepas de *Schistosoma mansoni* por morfometria de vermes adultos provenientes de infecção unissexual. Rev Soc Bras Med Trop 36(6):755–757

Magalhães LA, Carvalho JF (1973) Estudo morfológico de *Schistosoma mansoni* pertencentes a linhagens de Belo Horizonte (MG) e de São José dos Campos (SP). Rev de Saúde Pública de São Paulo 7:289–294

Martinez EM, Neves RH, Oliveira RMF, Machado-Silva JR, Rey L (2003) Características biológicas e morfológicas de cepas brasileiras de *Schistosoma mansoni* em *Mus musculus*. Rev Soc Bras Med Trop 36(5):557–564

Meuleman EA, Lyaruu DM, Khan MA, Holzmann PJ, Sminia T (1978) Ultrastructural changes in the body wall of *Schistosoma mansoni* during the transformation of the miracidium into the mother sporocyst in the snail host Biomphalaria pfeifferi. Z Parasitenkd 56(3):227–42

Miller FH, Tulloch GS, Kuntz RE (1972) Scanning electron microscopy of integumental surface of *Schistosoma mansoni*. J Parasitol 58:693–698

Nakano T, Fujino T, Washioka H, Tonosaki A, Goto K, Fried B (2003) Tegumentary papillae of *Echinostoma caproni* cercariae (Trematoda: Echinostomatidae). Parasitol Res 89:446–450

Neves RH, Pereira MJS, Oliveira RMF, Gomes DC, Machado-Silva JR (1998) *Schistosoma mansoni* Sambon, 1907: morphometrics differences between adult worms from sympatric rodent and human isolates. Mem Inst Oswaldo Cruz 93(1):309–312

Neves RH, Oliveira AS, Machado-Silva JR, Coutinho EM, Lenzi HL, Gomes DC (2002) Phenotypic characterization of *Schistosoma mansoni* adult worms recovered from undernourished mice: a morphometric study focusing on the reproductive system. Rev Soc Bras Med Trop 35:405–407

Neves, DP, Melo, AL, de Linardi, PM, Vitor, RWA (2005) *Schistosoma mansoni*. In: Atheneu (ed) Parasitologia Humana, 11rd edn. São Paulo, Brasil, pp 193–212

Oliveira AS, Barbosa AA Jr, Gomes DC, Machado-Silva JR, Barros AF, Neves RH, Coutinho EM (2003) Morphometric study of *Schistosoma mansoni* adult worms recovered from undernourished infected mice. Mem Inst Oswaldo Cruz 98(5):623–627

Paraense WL, Corrêa LR (1981) Observations on two biological races of *Schistosoma mansoni*. Mem Inst Oswaldo Cruz 76:287–291

Palombi A (1940) Gli stadi larvali del Trematodi del Golfo di Napoli. 3 contributo allo studio della morfologia, biologia e sistematica delle *cercarie marine*. Riv Parassitol 4:1–25

Pearce EJ, MacDonald AS (2002) The immunobiology of Schistosomiasis. Nat Reviews 2:499–511

Pereira ASA, Padilha RJR, Lima-Filho JL, Chaves MEC (2011) Scanning electron microscopy of the human low-density lipoprotein interaction with the tegument of *Schistosoma mansoni*. Parasitol Res 109:1395–1402

Rey L (2002) Schistosoma mansoni. In: Koogan G (ed) Bases da parasitologia médica, 2nd edn. Brasil, Rio de Janeiro, p 379

Robson RT, Erasmus DA (1970) The ultrastructure, based on stereoscan observations, of the oral sucker of the cercaria of *Schistosoma mansoni* with special reference to penetration. Z Parasitenkd 35:76–86

Rouquayrol, MZ, Filho, NA (2003) Epidemiologia e Saúde. In: Medsi (ed) Epidemiologia e Saúde, 6rd edn. Rio de Janeiro, Brasil

Russell-Pinto F, Bartoli F (2002) *Cercaria sevillana* n. sp., a new cercaria (Digenea: Microphallidae) from *Nassarius reticulatus* (L.) (Mollusca: Prosobranchia) in Portugal. Syst Parasitol 53:175–182

Schneider O, Zelck UE (2001) Differential display analysis of hemocytes from schistosome-resistant and schistosome-susceptible intermediate hosts. Parasitol Res Berlin 87(6):489–491

Senft AW, Gibler WB (1977) *Schistosoma mansoni* tegumental appendages: scanning microscopy following thiocarbohydrazide-osmium preparation. AmJTrop Med Hyg 26:1169–1177

Sobhon P, Koonchomboon T, Yuan HC, Upatham ES, Saitongdee P, Krautrachue M, Bubphaniroj P, Vongpayabal P (1986) Comparison of the surface morphology of adult *Schistosoma japonicum* (Chinese, Philippine and Indonesian strains) by scanning electron microscopy. Intern J Parasitol 16(3):205–216

Soliman GN, El Assal FM, Mansour NS, Garo K (1986) Comparison of two Egyptian strains of *Schistosoma mansoni* in hamsters. Z Parasitenkd 72:353–363

Southgate VR, Knowles RJ (1977) On *Schistosoma margrebowiei* Le Roux, 1933: the morphology of the Egg, Miraeidium and Cercaria, the compatibility with Speeies *of Bulinus*, and development in *Mesocricetus auratus*. Z Parasitenk 54:233–250

Southgate VR, Ross GC, Knowles RJ (1981) On Schistosoma leiperi Le Roux, 1955: scanning electron microscopy of adult worms, compatibility with species of Bulinus, development in *Mesocricetus auratus*, and isoenzymes. Z Parasitenkd 66:63–81

Souza FPC, Vitorino RR, Costa AP, Faria-Junior FC, Santana LA, Gomes AP (2011) Esquistossomose mansônica: aspectos gerais, imunologia, patogênese e história natural. Rev Bras Clin Med São Paulo 9(4):300–307

Storey DM, Ogbogu VC (1991) Observations on third-stage larval and adults of *Litomosoides carinii* (Nematoda: Filarioidea) by scanning and transmission electron microscopy. Ann Trop Med Parasitol 85:111–121

Van der Werf M, Vlas SJ, Broker S, Looman CWN, Nagelkerke NJD, Habbema JDF, Engels D (2003) Quantification of clinical morbidity associated with schistosome infection in sub-Saharan Africa. Acta Trop 86:125–139

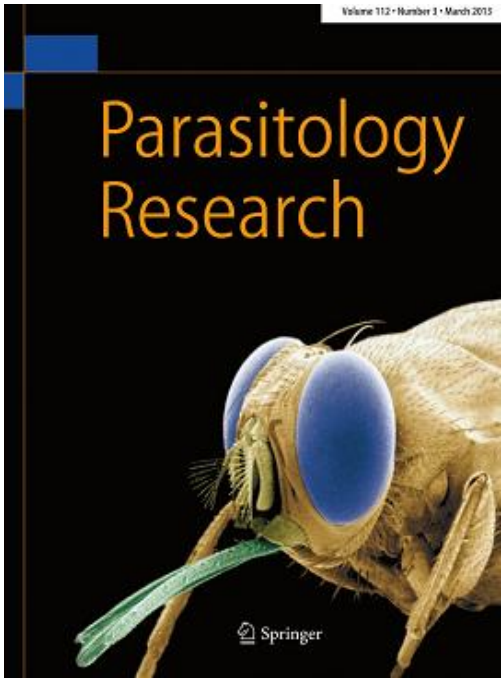
Van Hellemond JJ, Retra K, Brouwers FHM, Bas WM, Balkom V, Yazdanbakhsh M, Shoemaker CB, Tielens AGM (2006) A function of the tegument of schistosomes: clues from the proteome and lipidome. Intern J Parasitol 36:691–699

Vitorino RR, Souza FPC, Costa AP, Faria-Junior FC, Santana LA, Gomes AP (2012) Esquistossomose mansônica: diagnóstico, tratamento, epidemiologia, profilaxia e controle. Rev Bras Clin Med São Paulo 10(1):39–45

WHO (2012) World Health Organization. Schistosomiasis, Media Centre Fact sheet January, 115

6. SCANNING ELECTRON MICROSCOPY OF THE HUMAN LOW-DENSITY LIPOPROTEIN INTERACTION WITH THE TEGUMENT OF *SCHISTOSOMA MANSONI*

Capítulo publicado na Revista: Parasitology Research



Parasitol Res (2011) 109:1395–1402
DOI 10.1007/s00436-011-2386-4

ORIGINAL PAPER

Scanning electron microscopy of the human low-density lipoprotein interaction with the tegument of *Schistosoma mansoni*

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Received: 16 March 2011 / Accepted: 6 April 2011 / Published online: 19 April 2011

Abstract The interaction between host molecules and *Schistosoma mansoni* has been regarded as a key feature for parasite survival. In this work, scanning electron microscopy was used to study the interaction of human low-density lipoprotein (LDL) with the tegument of the adult worm of *S. mansoni*. Worms were incubated in RPMI 1640 containing 10% of LPDS and 40 μg LDL/mL during 30, 60, and 120 min. Control worms were processed in the same way, without LDL. After the incubations, the samples were fixed and processed to scanning electron microscopy. The results demonstrated interaction of the LDL particles with the male parasite tegument. Male and female worms incubated without LDL from 0 (control) to 120 min did not show alterations in the tegument. It was observed a larger number of LDL particles on the dorsal region of male adult worm than others regions (anterior, posterior and gynecophoral canal). The female tegument did not show adherence of LDL. Aggregates on the tegument of the male worm were in greater number and size in the incubation times of 30 and 60 min than 120 min. The comparison between 30 and 120 min of incubation showed that the particles' size diminished from 2,650–860 nm to 634–363 nm, respectively. Such reduction can be due to the capture and the use of the lipids by the worm. Therefore, the internalization of lipids from LDL by the male worms seems to be a mechanism independent of endocytosis. Differences between males and females suggest lipid transference from male to female through gynecophoral canal.

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Introduction

Schistosomes are human parasitic flatworms that constitute an important public health problem in many countries around the world. Human schistosomiasis is one of the most important of the neglected tropical diseases and is the second most prevalent tropical disease (Attallah et al. 2008; Abdul-Ghani et al. 2009; Mulvenna et al. 2010). Adult parasites live in the bloodstream from where they import nutrients across their body surface (the tegument; Krautz-Peterson et al. 2007).

The longevity of adult worms of *Schistosoma mansoni* can be considered as depending on two factors, a successful adjustment to the environmental conditions in the mesenteric veins of the host and an effective strategy for evasion of the immune system (Tempone et al. 1997).

Although the adult worms display a functional gut which is involved in the ingestion and digestion of whole cells and presumably the macromolecules, there is an intense traffic of small molecules through the tegument of these organisms. The tegument is an enigmatic structure—a double bilayer—forming the interface between parasite and host that has a vital role in the defense function to escape the immune response and the acquisition of nutrients, including carbohydrates, amino acids, lipids, lipoproteins, and other nutrients of similar size, as well as the excretion of catabolic products such as lactic acid (Tempone et al. 1997; Bryant 1993; Abath and Werkhauser 1996; Jones et al. 2004; Gan et al. 2006; Xiao et al. 2010).

The lipids for all of schistosome's membranes, including the tegumental membranes, must be derived from the host because the parasite does not synthesize cholesterol and fatty acids de novo (Bennett and Caulfield 1991). This way, they require a transport system for lipids. Multiple studies have shown the ability of the parasite membranes to bind host LDL (Rumjanek et al. 1988; Chiang and Caulfield 1989a, b; Tempone et al. 1997; Reis et al. 2006).

The LDL molecules bound to the external surface of the tegument, presumably through LDL-like receptors, conferring an extra advantage to the schistosomes by masking parasite antigens from being recognized by humoral or cell-mediated mechanism. It was previously shown that the binding of LDL to the surface of schistosomula inhibits the binding of human anti-schistosomal antibodies (Rumjanek et al. 1988; Chiang and Caulfield 1989a, b; Furlong et al. 1992; Dinguirard and Yoshino 2006).

Lipoproteins in human serum, purified LDL, very low-density lipoprotein, and apolipoprotein B (apo B) were demonstrated on the surface of schistosomula of *S. mansoni* by fluorescence and immunoelectron microscopy using a polyclonal goat anti-human apolipoprotein B antibody (anti-apo B; Chiang and Caulfield 1989a, b; Xu and Caufield 1992). The specific binding of human LDL to the schistosomula is mediated by GPI-linked low-molecular-weight proteins, which are continually synthesized and transported to the parasite surface (Xu and Caufield 1992). Studies developed in the schistosomula demonstrated that the parasite express a protein of 45 kDa when in contact with the human serum (Rumjanek et al. 1983). In addition, adult worms of *Schistosoma japonicum* presented a supposed receptor for human LDL, with apparent molecular weight of 43 kDa present in the parasite tegument and gut (Rogers et al. 1989).

The literature does not present many studies about interaction of LDL with the adult worm of *S. mansoni*. In this work, LDL particles were observed on the surface of the parasite tegument using scanning electron microscopy, mainly on the dorsal region of the male worm. However, on the tegument of female, there were not sites for LDL binding. Scanning electron microscopic has allowed the identification of ultrastructural features, tegumental alterations included swelling, fusion of tegumental ridges, vacuolisation, peeling, erosion, and sometimes the collapse of the tegument of diverse species between *S. mansoni* (Shuhua et al. 2002). The tegument of the *S. mansoni* constitutes an interface in the relationship of host–parasite, being an important target for the vaccine development and anti-helmintic drugs. Indeed, a number of vaccine antigens with high efficacy against schistosomes have been reported from the tegument membrane, including the tetraspanins (Tran et al. 2006) and others (Mulvenna et al. 2010; McManus and Loukas 2008).

Materials and methods

Snail

The freshwater snails *Biomphalaria glabrata* were obtained in the Malacology Unit, Aggeu Magalhães Research Center—FIOCRUZ, Recife, Brazil. The maintenance of the life cycle of *S. mansoni* was done in the Laboratorio de Imunopatologia Keizo Asami—LIKA in the Federal University of Pernambuco, Recife, Brazil.

Mice

Mice (*Mus musculus*) were obtained at 7–9 weeks of age, weighing 35–45 g. The animals were housed in cages (30×20×13 cm) containing sterile wood shaving bed. Standard diet (Labina®, Ralston Purina Ltda, São Paulo, Brazil) and water were available ad libitum. Room temperature was kept at 22±2°C and a 12:12-h light–dark cycle. Mice were infected individually with 100 *S. mansoni* cercariae, under artificial light for at least 2 h. Sixty days post-infection, the animals were sacrificed and the adult worms were collected by the perfusion of the hepatic portal system with saline solution (0.85% of NaCl, *p*/*v*; Duvall and Dewitt 1967). The worms were washed several times with PBS, pH 7.4, and fixed for scanning electron microscopy.

This study was approved by the ethical committees for research with animals of the Federal University of Pernambuco, Brazil.

Isolation of LDL

Sera from non-parasitized individuals were obtained from volunteers, after reading and signing the terms of agreement, and were submitted for differential ultracentrifugation for the LDL isolation in accordance with Havel et al. (1955).

Scanning electron microscopic examination

Immediately after the withdrawal, worms were divided in seven groups, each one with five parasites. The worms were incubated for 0, 30, 60, and 120 min, in culture plate (24 wells), with 1 ml of RPMI 1640 medium, containing 10% lipoprotein-free serum (LPDS) and 40 μg of LDL/mL. Controls without LDL were also included. After incubation, adult worms were fixed in 2.5% (*v*/*v*) glutaraldehyde in sodium cacodylate buffer (0.1 mol/L, pH 7.4) at 4°C and post-fixed with 1% (*w*/*v*) osmium tetroxide for 1 h. They were dehydrated through a graded series of ethanol, dried in a Hitachi HCP-2 critical point dryer machine using liquid carbon dioxide as a transitional medium. After drying, they were mounted on aluminum stubs and coated with gold in an ion-sputtering apparatus, FINE-COAT 1100—JEOL (Ion

Sputter JFC-1100), at 1.2 kV, 10 mA for 6 min. They were examined and photographed in the Scanning Electron Microscopy JEOL-JSM-5600 LV (Tokyo, Japan) Electron Microscope operating at 10 kV.

Results

Viability of the adult worms The medial dorsal region of the *S. mansoni* adult male worms incubated in RPMI 1640 without the presence of LDL, at the incubation times of 0, 30, 60, and 120 min (Fig. 1), did not show morphological alteration in the surface of the worms due to the prolonged time of incubation. It was also observed that the incubation of the worms with human LDL did not change, neither did the morphology nor the capacity of the male worm to interact with the female in the gynecophoral canal (Fig. 2 (5)). The Fig. 2 (6 and 7) shows the well-preserved structures of ventral and oral suckers, respectively, after 60 min of incubation with LDL in RPMI 1640 medium. The central and the intermediate zones, as well as the edge of the ventral sucker, are seen in Fig. 2 (8). They do not differ from the same structures of worms incubated in RPMI 1640 medium without LDL.

Interaction of the LDL with the tegument of the S. mansoni Scanning electron microscopy provides a high resolution method for examining the surface topography of the adult *S. mansoni*. The study of the tegument in the three main regions (anterior, medial, and posterior) of the adult male worms of the *S. mansoni* demonstrated the marked differences between them. The analysis of the material after incubation for 30, 60, and 120 min showed LDL particles bound to the surface of the tegument in the anterior, medial, and posterior regions of the male adult worm of *S. mansoni* (Fig. 3 (9–12)). It is worthwhile to observe the distribution of the LDL particles around the tubercles on the ridges and groves and the increased number of particles in the dorsal medial and posterior region, when compared with the anterior region. Lipoprotein binding on spines was not observed.

A greater number of LDL particles were seen on the dorsal regions of male adult worm of *S. mansoni* than in the ventral ones. The aggregates of LDL particles were noticed on the tegument in the incubation times of 30 (Fig. 4 (13 and 14)) and 60 min. Those aggregates have diminished when the incubations were done during 120 min (Fig. 4 (15 and 16)). The comparison between 30 and 120 min of incubation showed that the particles' sizes have also diminished from 2,650–860 nm to 634–363 nm, respectively. The presence of numerous porous on the tegument surface, which may act in the absorption of nutrients, is noticed.

The posterior region of the tegument presents a more furrowed aspect than the other regions of the worm with a bigger concentration of tubercles and spines (Fig. 5 (17)). Bulges are found between the grooves, suggesting the growth of new tubercles (Fig. 5 (17 and 18)). In this region, intense interaction of lipoproteins with the surface of the worm as seen in the medial region was not observed.

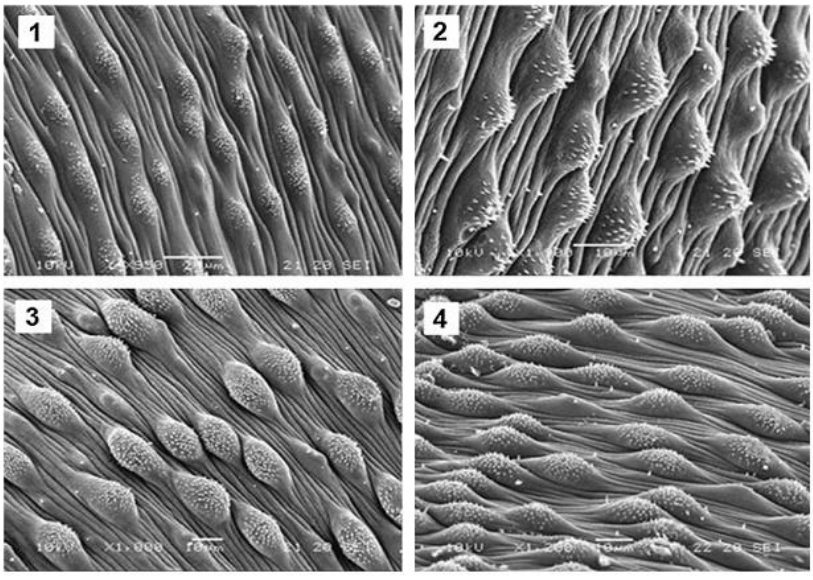

Fig. 1 *1–4* Electron micrographs of the medial dorsal region in the *Schistosoma mansoni* tegument. As observed, the tegument was completely preserved in the tubercles, spines, ridges, and grooves. Male adult worms were incubated with RPMI 1640 medium, supplemented with LPDS. *1* The medial dorsal region in the *S. mansoni* tegument incubated for 0 min (control; *Bar*=20 μm). *2* The medial dorsal region in the *S. mansoni* tegument incubated for 30 min (*Bar*=10 μm). *3* The medial dorsal region in the *S. mansoni* tegument incubated for 60 min (*Bar*=10 μm). *4* The medial dorsal region in the *S. mansoni* tegument incubated for 120 min (*Bar*=10 μm)

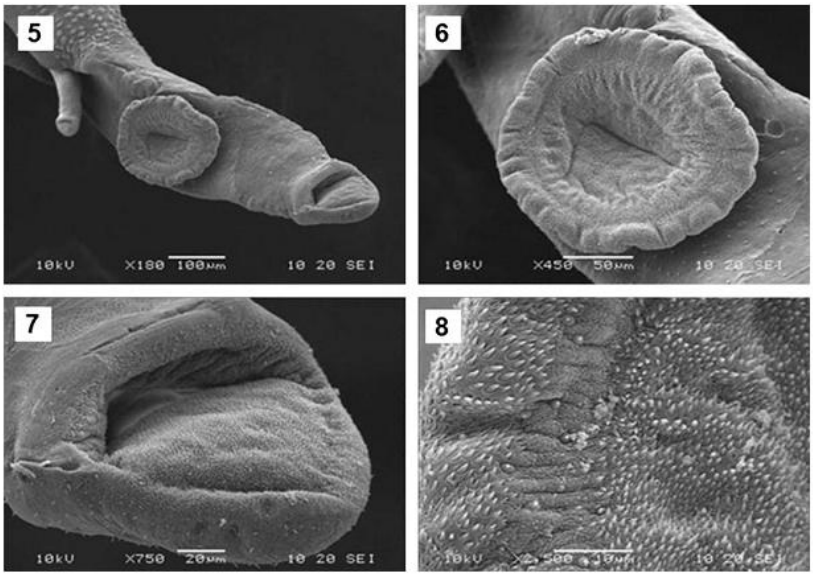

Fig. 2 *5–8* Ultrastructure of the anterior region and the suckers of the male adult worm of *Schistosoma mansoni* incubated with LDL for 60 min. *5* Anterior region with oral and ventral suckers, observing still a female in the gynecophoral canal (*Bar*=100 μm). *6* Ventral sucker (*Bar*=50 μm). *7* Oral sucker (*Bar*=20 μm). *8* Detail of the ventral sucker re-covered by a layer of spines (*Bar*=10 μm)

The gynecophoral canal of the male adult worm houses the female for reproduction. Small spines emerge from innumerable porous constituting a uniform layer (Fig. 5 (19)). It is possible to observe that the bigger the amount of spines and tubercles in the surface of the worm, the smaller will be the interaction with the lipoprotein particles. A small quantity of LDL in the gynecophoral canal with incubation of 120 min (Fig. 5 (20)) was seen.

The medial region of female tegument was smoother, without the presence of spines or tubercles (Fig. 6 (21)). The tail presented little spines, although quite different from males (Fig. 6 (24)). The analysis of the electron micrographs after the incubation times of 30, 60 and 120 min showed the absence of particles of LDL on the surface of the tegument in all the regions of the female adult worm of *S. mansoni* (Fig. 6 (22–24)).

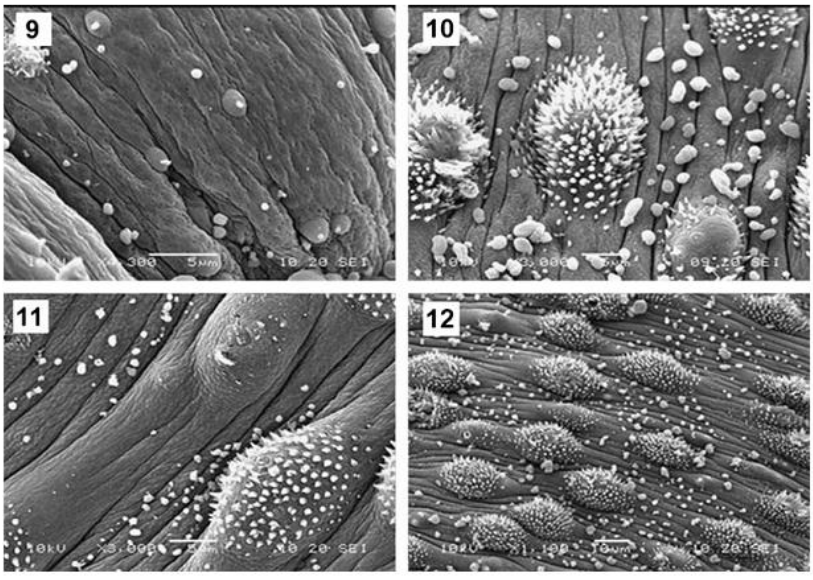

Fig. 3 *9–12* Electron micrographs of the tegument of the adult worms of *Schistosoma mansoni* incubated with human LDL for 30 min. LDL particles are seen on the surface of the tegument. *9* Dorsal area of the anterior region (*Bar*=5 μm). *10* Dorsal area of the medial region (*Bar*=5 μm). *11* Dorsal area of the medial region (*Bar*=5 μm). *12* Dorsal area of the posterior region (*Bar*=10 μm)

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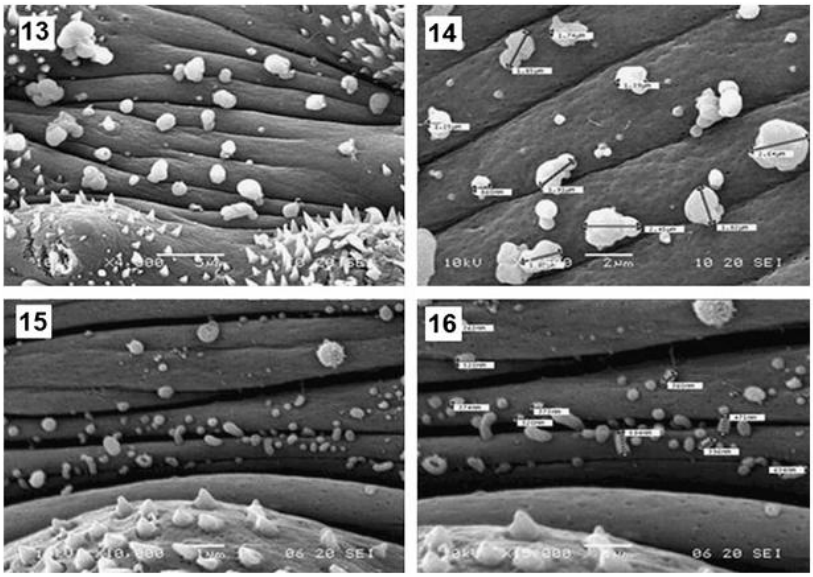

Fig. 4 *13–16* Electron micrographs of dorsal regions of *Schistosoma mansoni* worms showing the sizes of LDL particles after the incubations. *13* Dorsal region of *S. mansoni* with 30 min incubation showing large LDL particles bound to the tegument (*Bar*=5 μm). *14* Dorsal region of *S. mansoni* with 30 min incubation showing particles' size ranging from 2,650 to 860 nm (*Bar*=2 μm). *15* Dorsal region of *S. mansoni* with 120 min incubation showing small LDL particles (*Bar*=1 μm). *16* Dorsal region of *S. mansoni* with 120 min incubation showing particles' size ranging from 634 to 343 nm (*Bar*=1 μm)

Discussion

Many binding molecules of the membrane of *Schistosoma* are reported as targets of the host immune response (Smithers et al. 1990). Some of these perform a variety of important physiological functions including transmembrane ion channels, enzymatic activities, and receptors for several molecules (Cesari et al. 2010).

The adult worm of *S. mansoni* does not possess some essential metabolic routes, which disable them to synthesize important compounds for its development. These parasites neither synthesize a long-chain fatty acid nor sterols. However, the literature (Bennett and Caulfield 1991; Tempone et al. 1997; Loukas et al. 2001; Fan et al. 2003) indicates that they are dependents on such molecules for the modulation of its membranes, which are in constant

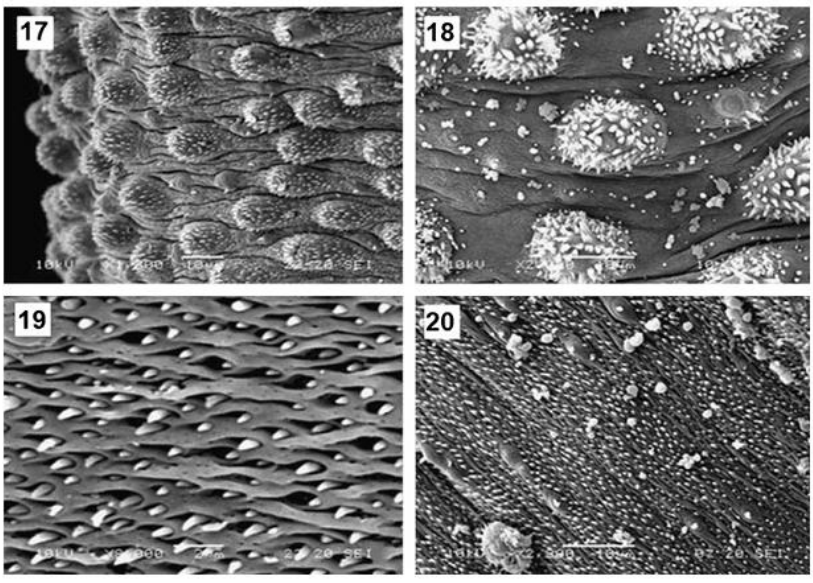

Fig. 5 *17–20* Scanning electron micrographs of the posterior region and gynecophoral canal of the adult male worm of *Schistosoma mansoni*. *17* Posterior region not presenting significant interaction of LDL particles, incubation of 60 min with LDL (*Bar*=10 μm). *18* Posterior region not presenting significant interaction of LDL particles, incubation of 60 min with LDL (*Bar*=10 μm). *19* Surface of the gynecophoral canal (*Bar*=2 μm). *20* Gynecophoral canal presenting a small number of LDL particles, 120 min of incubation (*Bar*=10 μm)

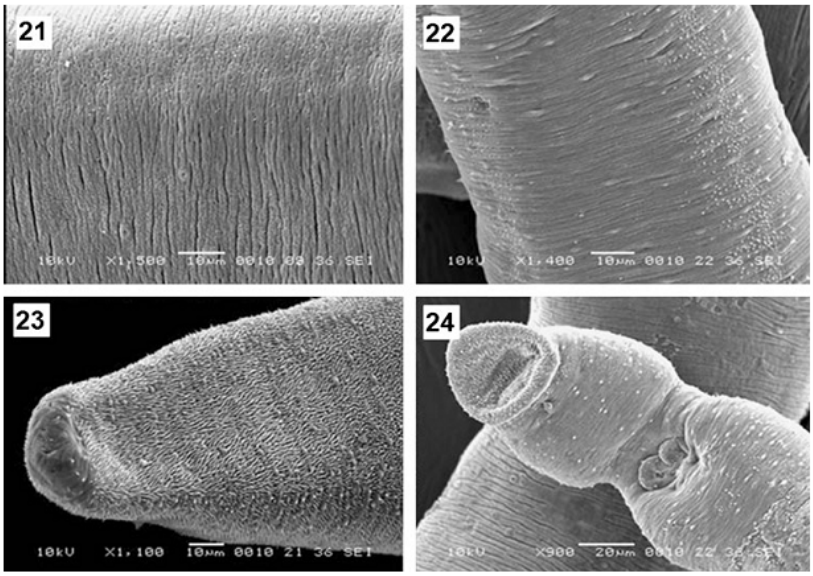

Fig. 6 *21–24* Electron micrographs of the tegument of female adult worms of *Schistosoma mansoni*. *21* Medial dorsal region in the female *S. mansoni* control tegument (*Bar*=10 μm). *22* Medial region with 60 min of LDL incubation (*Bar*=10 μm). *23* Posterior region with 120 min of LDL incubation not presenting interaction of particles (*Bar*=10 μm). *24* Anterior region with oral and ventral suckers with 60 min of incubation, observing the absence of LDL particles (*Bar*=20 μm)

changes. Thus, it is evident that the mechanisms for transference of lipids or precursors of lipids between the definitive host and the parasite must exist. This ability of the parasite to rapidly internalize molecules at its surface has implications for the development of vaccines.

The incapacity to synthesize fatty acids and cholesterol, the presence of these lipids in their membranes, the constant renovation of such membranes, and the relatively long permanence of the worm in the host organism have become the interest of many researchers in studying the origin of such molecules (Chiang and Caulfield 1989a, b; Furlong et al. 1992; Rogers 1991; Krieger and Herz 1994; Brouwers et al. 1998; Fan et al. 2003; Mulvenna et al. 2010).

The transport process across parasite cell membranes assume important role since these organisms have to compensate their biosynthetic deficiencies incorporating a variety of metabolites and precursors (Rumjanek et al. 1985). Thus, in the case of *Schistosoma*, the solute traffic across the tegument must be very intense during all stages of the parasite development after infection in human and other mammalians. Although the adult parasites possess a primitive gut where red cells can be digested, small molecule adsorption occurs through the tegument (Uglem and Read 1975; Rumjanek et al. 1985).

In this work, human LDL bound to the tegument of adult male worms of *S. mansoni* was investigated. As previously noted, there was no structural alteration of the spines and tubercles on the surface of the parasites caused by incubation with LDL, compared with studies involving drugs administration such as praziquantel and artemether that cause swelling, erosion, peeling, vesiculation, and collapse of tegumental ridges (Shuhua et al. 2002; Neves et al. 2010). Long-term LDL incubations (120 min) caused a decrease of approximately 30% of the original particles' size in the beginning of the experiments. The time required to stimulate the expression of LDL-binding protein on the surface of the adult worm is very short (Rumjanek et al. 1985; Rumjanek et al. 1988), causing the immediate capture of lipids. Our results bear the opinions of other authors (Xu and Caufield 1992) concerning the capture and the internalization of lipids by the *Schistosoma*, which seems to be independent of endocytosis. Thus, without endocytosis the incorporation of lipids occurs as the direct transference enters the LDL and the membrane of the parasite (Tempone et al. 1997). However, the possibility of LDL internalization in *S. mansoni* through LDL receptors in a way similar or not to human receptors cannot be excluded, as seen by Dinguirard and Yoshino (2006) who found LDL internalization through endocytosis in the larval stage of *S. mansoni*. Therefore, the characterization of tegument proteins is relevant to the improvement of the functional understanding of this structure and the identification of molecules that may act as targets for protective immune responses or may be useful in diagnosis (Abath et al. 2000; Cesari et al. 2010).

The literature demonstrates that many parasites request host lipoproteins for their survival. One of the first reports about the request of host lipoproteins by parasites came from a study with *Trichomonas vaginalis* (Peterson and Alderete 1984). The axenic culture of trypanosomes was dependent upon supplementation with the various

serum components for optimal parasite growth, particularly lipoproteins (Rogers 1991). For trypanosomes, a lipid-associated protein, apolipoprotein-I is responsible for parasite lysis (Coppens et al. 1987, 1988). It is possible that some components of the human lipid profile are also responsible for the natural resistance to schistosomes in host organism. Such a component might be low, defective, or less efficient in patients with lower LDL concentration (Vanhamme et al. 2003).

The LDL particle distribution on the male adult worm of *S. mansoni* was not uniform, being more concentrated on the dorsal medial region; meanwhile in the anterior and posterior regions, they were scarcely distributed. The gynecophoral canal is considered a contact zone male–female and can also serve to transfer free amino acids and other small molecules through easy diffusion (Senft and Gibler 1977); however, it does not seem to capture lipids through the mechanism of LDL binding, probably due to the absence of receptors in this region.

The presence of many aggregates of LDL may have been caused by the ability of the lipoproteins to self-assemble into homogeneous particles (Shih et al. 2007). The manner through which lipoprotein particles assemble has not yet been understood. Computational simulation has been used successfully to explain this assembly of micelle around the membrane (Bond et al. 2005; Psachoulia et al. 2006). An increase of small dense LDL has been shown in the abdominal adiposity and with the use of an oral contraceptive (de Graaf et al. 1993; Rizzo et al. 2003; Berneis and Rizzo 2004).

This study demonstrated that the binding of LDL to the tegument of male worms of *S. mansoni* is not uniform, being stronger in the dorsal region, suggesting a greater exposure of binding sites in this area. However, the absence of LDL interaction to the membrane surface in female worms may indicate a direct lipid transfer through the gynecophoric channel of male to female. Further studies should be made seeking a better understanding of these facts.

Acknowledgments This work was financially supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). The authors would like to thank Mr. Felipe Viegas and Mr. Sérgio Santos for their technical assistance.

References

Abath FG, Werkhauser RC (1996) The tegument of *Schistosoma mansoni*: functional and immunological features. Parasite Immunol 18:15–20

Abath FGC, Xavier EM, Allen R, Gomes YM, Lucena-Silva N, Baliza M, Simpson AJG (2000) Characterization of Sm13, a tegumental antigen of *Schistosoma mansoni*. Parasitol Res 86:745–752

Abdul-Ghani R, Loutfy N, El Sahn A, Hassan A (2009) Current chemotherapy arsenal for schistosomiasis mansoni: alternatives and challenges. Parasitol Res 104:955–965

Attallah AM, Wahba MA, Elsheikha HM, Abbas AT, Abdel Aziz MM, El-Hemaly MA (2008) Outcomes of *Schistosoma mansoni* infection in outbred albino mice exposed to Larvin contaminant. Parasitol Res 103:567–576

Bennett MW, Caulfield JP (1991) Specific binding of human low-density lipoprotein to the surface of *Schistosoma mansoni* and ingestion by the parasite. Am J Pathol 138:1173–1182

Berneis K, Rizzo M (2004) LDL size: does it matter? Swiss Med Wkly 134:720–724

Bond PJ, Curhbertson J, Sansom MSP (2005) Simulation studies of the interactions between membrane proteins and detergents. Biochem Soc Trans 33:910–912

Brouwers JFHM, Van Hellemond JJ, Van Golde LMG, Tielens AGM (1998) Ether lipids and their possible physiological function in adult *Schistosoma mansoni*. Mol Biochem Parasitol 96:49–58

Bryant C (1993) Organic acid excretion by helminths. Parasitol Today 9(2):58–60

Cesari IM, Ballen DE, Mendoza L, Ferrer A, Pointier JP, Kombila M, Richard-Lenoble D, Théron A (2010) Immunoblot analysis of membrane antigens of *Schistosoma mansoni*, *Schistosoma intercalatum*, and *Schistosoma haematobium* against Schistosoma-infected patient sera. Parasitol Res 106:1225–1231

Chiang C-P, Caulfield JP (1989a) Human lipoprotein binding to schistosomula of *Schistosoma mansoni*. Displacement by polyanions, parasite antigen masking, and persistence in young larvae. Am J Pathol 135:1015–1024

Chiang C-P, Caulfield JP (1989b) The binding of human low-density lipoproteins to the surface of schistosomula of *Schistosoma mansoni* is inhibited by polyanions and reduces the binding of anti-schistosomal antibodies. Am J Pathol 134:1007–1017

Coppens I, Opperdoes F, Courtoy PJ, Baldhuin P (1987) Receptor mediated endocytosis in the bloodstream form of Trypanosoma brucei. J Protozool 34:465–473

Coppens I, Baudhuin P, Opperdoes FR, Courtoy PJ (1988) Receptors for the host low density lipoproteins on the hemoflagellate Trypanosoma brucei: purification and involvement in the growth of the parasite. Proc Natl Acad Sci USA 85:6753–6757

de Graaf J, Swinkels DW, Demacker PN, de Haan AF, Stalenhoef AF (1993) Differences in the low density lipoprotein subfraction profile between oral contraceptive users and controls. J Clin Endocrinol Metab 76:197–202

Dinguirard N, Yoshino TP (2006) Potential role of a CD36-like class B scavenger receptor in the binding of modified low-density lipoprotein (acLDL) to the tegumental surface of *Schistosoma mansoni* sporocysts. Mol Biochem Parasitol 146:219–230

Duvall RH, Dewitt WB (1967) An improved perfusion technique for recovering adults schistosomes from laboratory animals. Am J Trop Med 16:483

Fan J, Gan X, Yang W, Shen L, Mcmanus DP, Brindley PJ (2003) A *Schistosoma japonicum* very low-density lipoprotein-binding protein. Int J Biochem Cell Biol 35:1436–1451

Furlong ST, Thibault KS, Rogers RA (1992) Fluorescent phospholipids preferentially accumulate in sub-tegumental cells of schistosomula of *Schistosoma mansoni*. J Cell Sci 103:823–830

Havel RJ, Eder HA, Bragdon JH (1955) The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. J Clin Invest 34:1345–1353

Jones MK, Gobert GN, Zhang L, Sunderland P, McManus DP (2004) The cytoskeleton and motor proteins of human schistosomes and their roles in surface maintenance and host parasite interactions. BioEssays 26:752–765

Krautz-Peterson G, Camargo S, Huggel K, Verrey F, Shoemaker CB, Skelly PJ (2007) Amino acid transport in schistosomes charac-

terization of the permease heavy chain SPRM1hc. J Biol Chem 282(30):21767–21775
Krieger M, Herz J (1994) Structure and functions of multiligand lipoprotein receptors: macrophage scavenger receptors and LDL receptor-related protein (LRP). Ann Rev Biochem 62:601–637
Loukas A, Jones MK, King LT, Brindley PJ, McManaus DP (2001) Receptor for Fc on the surfaces of schistosomes. Infect Immun 69:3646–3651
McManus DP, Loukas A (2008) Current status of vaccines for schistosomiasis. Clin Microb Rev 21:225–242
Mulvenna J, Moertel L, Jones MK, Nawaratna S, Lovas EM, Gobert GN, Colgrave M, Jones A, Loukas A, McManus DP (2010) Exposed proteins of the *Schistosoma japonicum* tegument. Int J Parasitol 40(5):543–554
Neves JKAL, Botelho SPS, Melo CML, Pereira VRA, Lima MCA, Pitta IR, Albuquerque MCPA, Galdino SL (2010) Biological and immunological activity of new imidazolidines against adult worms of *Schistosoma mansoni*. Parasitol Res 107(3):531–538
Peterson KM, Alderete JF (1984) Selective acquisition of plasma proteins by *Trichomonas vaginalis* and human lipoproteins as a growth requirement for this species. Mol Biochem Parasitol 12:37–48
Psachoulia E, Bond PJ, Sansom MSP (2006) MD simulations of mistic: conformational stability in detergent micelles and water. Biochemistry 45:9053–9058
Reis EAG, Reis MG, Silva RCR, Carmo TMA, Assis AMO, Barreto ML, Parraga IM, Santana MLP, Blanton RE (2006) Biochemical and immunologic predictors of efficacy of treatment or reinfection risk for *Schistosoma mansoni*. Am J Trop Med Hyg 75 (5):904–909
Rizzo M, Barbagallo CM, Severino M, Polizzi F, Onorato F, Noto D (2003) Low-density-lipoprotein peak particle size in a Mediterranean population. Eur J Clin Invest 33:126–133
Rogers MV (1991) Do parasites express receptors for host lipoproteins? Parasitol Today 7:117–120
Rogers MV, Henkle KJ, Fidge NH, Mitchell GF (1989) Identification of a multispecific lipoprotein receptor in adult *Schistosoma japonicum* by ligand blotting analyses. Mol Biochem Parasitol 35:79–88
Rumjanek FD, Mclaren DJ, Smithers SR (1983) Serum-induced expression of a surface protein in *Schistosoma mansoni*: a possible receptor for lipid uptake. Mol Biochem Parasitol 9:337–350
Rumjanek FD, Pereira MAC, Silveira AMV (1985) The interaction of human serum with the surface membrane of Schistosomula of *Schistosoma mansoni*. Mol Biochem Parasitol 14:63–73
Rumjanek FD, Campos EG, Afonso LC (1988) Evidence for the occurrence of LDL receptors in extracts of schistosomula of *Schistosoma mansoni*. Mol Biochem Parasitol 28:145–152
Senft AW, Gibler WB (1977) *Schistosoma mansoni* tegumental appendages: scanning microscopy following thiocarbohydrazide–osmium preparation. Am J Trop Med Hyg 26:1169–1177
Shih AY, Freddolino PL, Arkhipov A, Schulten K (2007) Assembly of lipoprotein particle revealed by coarse-grained molecular dynamics simulations. J Struct Biol 157:579–592
Shuhua X, Binggui S, Utzinger J, Chollet J, Tanner M (2002) Ultrastructural alterations in adult Schistosoma mansoni caused by artemether. Mem Inst Oswaldo Cruz 97(5):717–724
Smithers SR, Hackett F, Braga V, Simpson AJG (1990) Immunoblotting identifies additional antigens recognized by mice protectively vaccinated with adult *Schistosoma mansoni* tegumental membranes. Parasitol Res 76:454–456
Tempone AJ, Bianconi ML, Rumjanek FD (1997) The interaction of human LDL with the tegument of adult *Schistosoma mansoni*. Mol Cell Biochem 177:139–144
Tran MH, Pearson MS, Bethony JM, Smyth DJ, Jones MK, Duke M, Don TA, McManus DP, Correa-Oliveira R, Loukas A (2006) Tetraspanins on the surface of Schistosoma mansoni are protective antigens against schistosomiasis. Nat Med 12:835–840
Uglem GL, Read CP (1975) Sugar transport and metabolism in *Schistosoma mansoni*. J Parasitol 61:390–397
Vanhamme L, Paturiaux-Hanocq F, Poelvoorde P, Nolan DP, Lins L, Van Den Abbeele J, Pays A, Tebabi P, Van Xong H, Jacquet A, Moguilevsky N, Dieu M, Kane JP, De Baetselier P, Brasseur R, Pays E (2003) Apolipoprotein L-I is the trypanosome lytic factor of human serum. Nature 422:83–87
Xiao S, Xue J, Shen B (2010) Transmission electron microscopic observation on ultrastructural alterations in *Schistosoma japonicum* caused by mefloquine. Parasitol Res 106:1179–1187
Gan X-X, S L-Y, Wang Y, Ding J-Z, Shen H-Y, Zeng X-P, McManus DP, Brindley PJ, Fan J (2006) Recombinant tegumental protein *Schistosoma japonicum* very low density lipoprotein binding protein as a vaccine candidate against *Schistosoma japonicum*. Mem Inst Oswaldo Cruz 101:9–13
Xu X, Caufield JP (1992) Characterization of human low density lipoprotein binding proteins on the surface of schistosomula of *Schistosoma mansoni*. Eur J Cell Biol 57:229–235

7. ISOFORMS OF HSP70-BINDING HUMAN LDL IN ADULT WORMS OF *SCHISTOSOMA MANSONI*

Capítulo enviado à Revista: Parasitology International

Manuscript number: PARINT-D-14-00033

Elsevier Editorial System(tm) for Parasitology International
Manuscript Draft

Manuscript Number:

Title: Isoforms of Hsp70-binding human LDL in adult worms of Schistosoma mansoni

Article Type: Research Paper

Keywords: Schistosoma; Schistosoma mansoni; Lipoproteins; LDL; Hsp70.

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Abstract: Schistosoma mansoni is one of the most important and prevalent parasites infecting humans. It gives rise to pathological changes that can result in physical incapacity or even in death. The worms are well adapted to the host and its longevity can be seen as a consequence of effective escape from the host's immune system. In the blood circulation, lipoproteins not only help to conceal the worm from the attack by the host antibodies, but also act as a source of lipids for the S. mansoni. Scanning electron microscopy experiments showed the presence of LDL particles on the surface of adult worms of S. mansoni, which decreased in size when the incubation time increased. In this study, transmission electron microscopy and proteomic analyzes were used to locate and identify in the S. mansoni binding proteins to human plasma LDL. Ultrathin sections of S. mansoni adult worms were cut transversely from the anterior, medial and posterior regions of the parasite. The grids were exposed to human serum diluted 1:10 for 90min at room temperature, followed by incubations with the chicken anti-LDL antibody diluted 1:1000 for 5h protein A conjugated to 20nm gold particles for 30min. The immunolabelling experiments revealed particles of gold in the tegument, muscle region and spine in male worms and around vitellin cells in the female. Two dimensional electrophoresis and immunoblotting using incubations with human serum, anti-LDL antibodies and anti-chicken IgY (IgG) peroxidase conjugate were done in an attempt to identify LDL binding proteins in the S. mansoni. The analysis of the binding proteins with LC-MS enabled the identification of two isoforms of the Hsp 70 chaperone in S. mansoni. Hsp 70 is involved in the interaction with apo-B in the cytoplasm and its transport to the endoplasmic reticulum. Further studies are needed to clarify the functional role of Hsp 70 in the S. mansoni, mainly related to the human LDL.

Cover Letter

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February, 5rd 2014

Dear Sirs,

We are submitting to the **Parasitology International** the manuscript entitles: **"Isoforms of Hsp70-binding human LDL in adult worms of *Schistosoma mansoni*"** to the appreciation for this journal. Our request is reasoned in the following aspects:

1. In Brazil, *Schistosoma mansoni* in the main agent of Schistosomiasis, which infects 12 millions of people, mainly in the Northeast of the country;
2. This parasite does not synthesize long fatty acids, but such molecules are found as components of their membranes, being acquired from the host's organism;
3. Host serum lipoproteins could be a source of lipids for the Schistosoma. In this work, we identified LDL-binding proteins in adult worms of *S. mansoni*;
4. Microscopic experiments allowed the location of the binding sites of the lipoprotein to the worm;
5. Furthermore, they also enabled a breakthrough in understanding the interaction parasite-host

None of the authors has any potential financial conflict of interest related to this manuscript.

Sincerely yours,

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***Highlights**

Research highlights

- *Schistosoma mansoni* infects 12 millions of people in Brazil.
- Majority of the infected patients live in the Northeast of the country.
- This parasite does not synthesize long fatty acids, but acquire from the host's organism.
- Human LDL can be a source of lipids for the Schistosoma.

Isoforms of Hsp70-binding human LDL in adult worms of *Schistosoma mansoni*

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Abstract

Schistosoma mansoni is one of the most important and prevalent parasites infecting humans. It gives rise to pathological changes that can result in physical incapacity or even in death. The worms are well adapted to the host and its longevity can be seen as a consequence of effective escape from the host's immune system. In the blood circulation, lipoproteins not only help to conceal the worm from the attack by the host antibodies, but also act as a source of lipids for the *S. mansoni.* Scanning electron microscopy experiments showed the presence of LDL particles on the surface of adult worms of *S. mansoni,* which decreased in size when the incubation time increased. In this study, transmission electron microscopy and proteomic analyzes were used to locate and identify in the *S. mansoni* binding proteins to human plasma LDL. Ultrathin sections of *S. mansoni* adult worms were cut transversely from the anterior, medial and posterior regions of the parasite. The grids were exposed to human serum diluted 1:10 for 90min at room temperature, followed by incubations with the chicken anti-LDL antibody diluted 1:1000 for 5h protein A conjugated to 20nm gold particles for 30min. The immunolabelling experiments revealed particles of gold in the tegument, muscle region and spine in male worms and around vitellin cells in the female. Two dimensional electrophoresis and immunoblotting using incubations with human serum, anti-LDL antibodies and anti-chicken IgY (IgG) peroxidase conjugate were done in an attempt to identify LDL binding proteins in the *S. mansoni*. The analysis of the binding proteins with LC-MS enabled the identification of two isoforms of the Hsp 70 chaperone in *S. mansoni*. Hsp 70 is involved in the interaction with apo-B in the cytoplasm and its transport to the endoplasmic reticulum. Further studies are needed to clarify the functional role of Hsp 70 in the *S. mansoni,* mainly related to the human LDL.

Keywords: Schistosoma; *Schistosoma mansoni*; Lipoproteins; LDL; Hsp70.

1. Introduction

Schistosomiasis is an important parasitic disease in tropical and subtropical countries [1-3] caused by trematode worms of the genus Schistosoma [4-5]. One of the three main species that infect humans is *Schistosoma mansoni*, which is found in sub-Saharan Africa, parts of the Middle East, Brazil, Venezuela and some Caribbean islands [6]. This parasite has a complex life cycle in invertebrate and vertebrate hosts, becoming sexually mature at approximately four weeks post-infection in humans and other susceptible animals [7]. The final habitat of the male and female adult worms is the mesenteric vasculature of the vertebrate host, where it remains in the bloodstream for decades, despite being permanently exposed to the host's immune system. They have an effective method for evading the immune system that depends on the properties of the parasite tegument [8-10].

The incorporation of lipids by the worms is an important element in determining the properties of the parasite membranes. However, schistosomes are incapable of synthetizing cholesterol and fatty acids [11]. Endogenous receptors may perform important functions, such as absorption of lipids from host plasma lipoproteins for use in growth, development, maintenance and synthesis of parasite membranes and also host-immune evasion through accumulation and display of host-lipids on the surface tegument [12-14].

Several studies have demonstrated the expression of proteins in the protozoa, helminthes, schistosomula and adult worms of Schistosoma, which are capable of binding to low density lipoprotein (LDL), very low density lipoprotein (VLDL) and apoprotein B (apo B) [12,14-17]. Through binding to apo B-100, the LDL receptor mediates the clearance of LDL in human plasma [18]. After the binding of the extracellular components on the cell surface receptors, clathrin vesicles are formed and these play an important role in the internalization of proteins and lipids [19].

Clathrin-mediated endocytosis in humans is a complex process involving accessory and regulatory proteins, including heat shock protein 70 (Hsp 70), a chaperone that has been the subject of extensive research because it is involved in a number of cellular processes [20-21]. It is responsible for disrupting clathrin-clathrin interactions, leading to uncoating [22]. Hsp 70 is a cytoplasmic protein with a property

of targeting the hydrophobic domains of substrate polypeptides, guiding proteins to cell organelles [18, 23-26]. Hsp 70 is express in some of the stages of the *S. mansoni* life cycle (miracidium, sporocyst, schistosomulum and adult worm) [27].

The present study identified two proteins belonging to the Hsp70 family, which bind to human LDL. Further studies are needed to shed light on the host-parasite relationship, in an attempt to identify the functional role of Hsp 70 in the lipid metabolism of *S. mansoni.*

2. Materials and methods

2.1 Reagents

Chicken anti-LDL affinity-purified polyclonal antibody (Chemicon International, New Jersey, USA). Agarose, coomassie blue, polyvinyldifluoride (PVDF) membranes, sodium dodecyl *sulfate (SDS),* urea, thiourea, 3-[(3-cholamidopropyl)–dimethylammonium]–propane-sulfonate (CHAPS), protease inhibitor mix, IPG buffer, 13cm linear IPG strips pH 3-10, tris (GE Healthcare, New Jersey, USA). Trypsin (Promega, Southampton, Hampshire, UK). Acetonitrile (ACN), trifluoroacetic acid (TFA), formic acid, ethanol, hydrochloric acid (HCl), and glycerol (Merck, Darmstadt, Germany). The other reagents used in the experiments were from Sigma-Aldrich, St. Louis, USA.

2.2 Maintenance of parasite

The SLM strain of *Schistosoma mansoni,* (São Lourenço da Mata, Brazil) was maintained in *Biomphalaria glabrata* snails and male Swiss mice. Animals aged 7-9 weeks, weighing 35-45 g, were housed in cages (30x20x13cm) containing a sterile bed of wood shavings. A standard diet (Labina®, Ralston Purina Ltda, São Paulo, Brazil) and water were made available *ad libitum*. The room temperature was kept at 22 ± 2°C and a 12:12 hour light–dark cycle was maintained. Mice were infected by exposure to a

S. mansoni cercariae suspension containing approximately 100 cercariae using the tail immersion technique [28]. After 8 weeks of infection, the *S. mansoni* adult worms were recovered by perfusion of the hepatic portal system [29]. The worms were stored at -20°C for crude extract preparations or fixed for electron microscopy experiments. The experimental protocols were in accordance with the requirements of the Animal Experiments Ethics Committee of the Federal University of Pernambuco, Brazil.

2.3 Transmission electron microscopy (TEM)

Freshly perfused adult parasites were fixed with 0.1 % (v/v) glutaraldehyde, 4 % (v/v) PFA in 0.1 M sodium cacodylate buffer pH 7.2, overnight, at 4 °C. Parasites were washed with the same cacodylate buffer and then dehydrated in a graded series of ethanol [30, 50, 70, 90 and 100 % (v/v), 20 min each], then infiltrated and embedded in L.R. White resin. The material was placed in gelatin capsules and the resin allowed to polymerize at 50 °C. Ultrathin sections were cut transversely from the anterior, medial and posterior regions of the parasite. Sections were subsequently incubated with blocking solution (1.5 % (w/v) BSA, 0.01 % (v/v) Triton 20 in PBS) for 40 min, at room temperature and washed in the same solution.

The grids were exposed to human serum diluted 1:10 in blocking solution for 90 min at room temperature, followed by washing in the same solution and incubation with the chicken anti-LDL antibody diluted 1:1000 in blocking solution for 5 h. Finally, the grids were incubated with protein A conjugated to 20 nm gold particles diluted 1:50 in PBS for 30 min. As a negative control, the sections were incubated only with protein A conjugated to colloidal gold particles.

2.4 Two dimensional electrophoresis (2DE)

Adult worms (100-200mg wet weight) were homogenized in 500 µL of PBS containing protease inhibitor and centrifuged at 3,000 x*g* for 30 min, at 4 °C. The supernatant was discarded and the pellet re-suspended in 500 µL of PBS plus protease inhibitor. After protein quantification [30], 400 µg of protein were lyophilized and re-

suspended in the rehydration solution (7 M urea, 2 M thiourea, 0.5 % (w/v) CHAPS, 0.2 % (w/v) DTT, 0.5 % (v/v) IPG buffer (pH 3-10) and 0.002 % (w/v) bromophenol blue).

For the first dimension, proteins were subjected to isoelectric focusing on 13 cm linear IPG pH 3-10 strips using a Multiphor II system (GE Healthcare, New Jersey, USA) under the following conditions: 300 V (1 min), 300-3500 V (1 h 30 min), 3500 V (4 h). Focused strips were incubated with 65 mM DTT in 10 mL equilibration buffer (50 mM (w/v) Tris/HCl, 6 M (w/v) urea, 30 % (v/v) glycerol, 2 % (w/v) SDS), followed by a further incubation with 135 mM iodoacetamide in the same buffer. Both incubations were conducted for 15 min. For the second dimension, the strips were directly applied to 10 % homogeneous SDS-PAGE and overlaid with 1 % (w/v) agarose solution in electrophoresis buffer. SDS-PAGE was performed with a constant current (20 mA per gel) using a vertical system for 4 h. Analytical gels were stained with coomassie blue. The pattern quality and reproducibility of each set of 2DE gel were evaluated by conducting three independent experiments.

2.5 Immunoblotting

Proteins resolved using SDS–PAGE were transferred to PVDF membranes at a constant current of 100 mA, for 1 h, followed by incubation with PBS containing 3 % (w/v) casein for 3h at 4°C. The membranes were incubated for 2h at 25 °C with human serum diluted 1:10, overnight at 4°C with anti-LDL antibody diluted 1:1000 and for 3h at 4°C with anti-chicken IgY (IgG) peroxidase conjugate diluted 1:1000. All dilutions were made with PBS. The membranes were also washed with PBS between the incubations. Proteins visualization was carried out using TMB substrate. The reaction was stopped by the addition of distilled water. Analysis of the 2DE electrophoresis gel and immunoblotting were conducted by digitization of the images caught in the Transluminator L-Pix HE Scanner (Loccus Biotecnologia, São Paulo, Brazil) and analyzed using Image Master 2D Platinum (GE Healthcare, New Jersey, USA).

2.6 Protein Identification

The selected protein spots from the 2DE analysis were excised from gels and destained using 200 μL of 25 mM NH_4HCO_3 in 50 % (v/v) ACN pH 8.0, and vortexed three times for 5-10 min each. The gels were washed with Milli-q water, the supernatants discarded and the samples dried in a Speed-Vac (Concentrator Plus, Eppendorf, Brazil). The dried gels were rehydrated overnight with 50 mM NH_4HCO_3 in the presence of trypsin solution (20 ng/μL), at 37 °C. The peptide fragments digested by trypsin were extracted with 50 % (v/v) ACN /5 % (v/v) TFA, desalted using a C18 reverse phase ZipTips and dried in a Speed-Vac. After this, 10 μL of each sample were injected into a nanoEase C18 (100 mm × 100 μm) column (Waters, Milford, MA, USA) and eluted (0.4 μL/min) with a linear gradient [10–50% (v/v)] of ACN containing 0.1 % (v/v) formic acid. Electrospray tandem mass spectra (ESI) were recorded using a Q-Tof quadrupole/orthogonal acceleration time-of-flight spectrometer (Waters, Milford, MA, USA) interfaced with the nano Acquity System capillary chromatograph. The ESI voltage was set at 3500 V using a metal needle, the source temperature was 100 °C and the cone voltage 40 V. Instrument control and data acquisition were conducted using a MassLynx data system (Version 4.1, Waters). Experiments were performed by scanning from a mass-to-charge ratio (*m*/*z*) of 200–2000 using a scan time of 1 s, applied during the whole chromatographic process.

The mass spectra corresponding to each signal from the total ion current (TIC) chromatogram were averaged, allowing for accurate measurement of molecular mass. The exact mass was determined automatically using Q-Tof's LockSpray™ (Waters, Milford, MA, USA). Data-dependent MS/MS acquisitions were performed on precursors with charge states of 2 and 3 over a range of 50–2000 *m*/*z* and below a 2 *m*/*z* window. A maximum of three ions were selected for MS/MS from a single MS survey. The adduct masses of Na^+ and K^+ were automatically excluded. Collision-induced dissociation (CID) MS/MS spectra were obtained using argon as the collision gas at a pressure of 13 psi, and the collision voltage varied between 18 and 45 V depending on the mass of the precursor.

The scan rate was 1 scan/s. All data were processed using the ProteinLynx Global server (version 2.0, Waters). The processing locks mass corrected the *m*/*z* scale

of both the MS and MS/MS data using the lock spray reference ion. The MS/MS data were also charge-state deconvoluted and deisotoped using a maximum entropy algorithm (MaxEnt 3, Waters).

Proteins were identified by correlation of tandem mass spectra and the NCBInr database of proteins (Version 050623, 2,564,994 sequences) using the MASCOT software (Matrix Science, version 2.1). They were assumed to be carbamidomethylated, and a variable modification of methionine (oxidation) was allowed. Identification was considered positive, if at least two peptides matched with a mass accuracy of less than 0.2 Da.

3. Results

3.1 Immunolabelling of S. mansoni tegument incubated with human serum

When ultrathin sections from the anterior, medial and posterior regions of the *S. mansoni* were incubated with human serum followed by incubations with anti-LDL antibody and protein-A-gold-complex, immunolabelling was found in various parts of the male and female worms (Fig. 1). In adult male parasites gold particles were found in the tegument, muscular region and spine, being more abundantly distributed in muscle fiber (Fig. 1B and 1C). Immunolabelling was detected in and around vitellin cells in the females (Fig. 1D). No reactivity was found in the male and female controls (Fig. 1A), in which incubations were performed with the protein-A-gold-complex alone, without the anti-LDL antibody step.

3.2 Recognition of LDL-binding proteins in S. mansoni via 2D-electrophoreisis

Two-dimensional electrophoresis of proteins from adult *S. mansoni* worms (Fig. 2A) and western blotting analysis (Fig. 2B) were carried out to identify LDL-binding proteins in the parasite. Coomassie-stained analytical gels revealed a total of 215 spots, of which five were identified by anti-LDL antibodies. The Image Master 2D Platinum Software created a matching algorithm that compared images of related spots. Parameters such as MW and pI were assigned to the images analyzed. The results

showed that spots recognized by the anti-LDL antibodies ranged from pI 5.8 to 6.3 and MW 71 to 73 kDa (Figs. 2A and 2B). The LDL-binding spots identified were numbered and located in the corresponding 2D electrophoresis gel (Fig. 2A).

3.3 LC MS/MS analysis of the proteins recognized by anti-LDL antibody

Spots that consistently registered in the same position in the gel and blotting were considered to be the same protein. The five spots were successfully identified by LC MS/MS. The tandem mass spectra generated by LC MS/MS were correlated with the NCBInr database using MASCOT software. Two different forms of the chaperone Heat shock protein 70 (Hsp70) of *S. mansoni* were identified (Table 1), showing high coverage and uniform distribution of amino acid. Partial sequences of the peptides were researched in the database with the help of the Blast bioinformatics program, resulting in a high degree of confidence in the proteins identified.

3 Discussion

Human schistosomiasis is often accompanied by perturbation of lipid homeostasis [31-32]. It is well known that *S. mansoni* does not synthesize fatty acids and sterols, but depends on such molecules for the formation of their membranes [11, 33]. To meet their metabolic needs and evade immune recognition, these parasites use host molecules such as serum lipoproteins, glycolipids from the blood groups, and histocompatibility antigens [8, 14, 34-39].

Evidences suggest that there are mechanisms for transferring lipid or lipid precursors between the definitive host and the parasite [14, 33, 36, 40]. LDL is rich in sterols and phosphatidylcholine, which are required for synthesis of membranes and also plays a major role in cholesterol transport in human plasma [16, 41]. Previous studies have shown that LDL can be linked to a receptor on the surface of the *S. mansoni* forming a protective cover on the parasite tegument [14, 16, 41-44]. The LDL receptor is a multi-domain protein and has been described in schistosomula and adult worms of *S. mansoni* and *S. japonicum* [16, 41, 33, 43]. The literature contains reports

of proteins that are synthesized constitutively and transported to the parasite surface at various stages in its life cycle [17, 43, 45, 46].

Previous study confirmed the interaction of LDL with the tegument of *S. mansoni* (SLM strain) using scanning electron microscopy. The size of LDL aggregates decreased over the incubation period (0-120 min) [47], and it was suggested that it occurred as a result of lipid transfer from the host lipoproteins to the worms.

The accumulation and release of lipids has different standards in male and female *S. mansoni* [48-51]. In females, the lipid accumulation was present only in vitelline cells [49]. Likewise, we found LDL-colloidal gold particles inside and around the vitelline cells, while in males they were present in the muscle cells, syncytium and spines. It has been reported that exposure of *S. mansoni* to human serum allows the LDL-linked protein to emerge on the surface [15].

There is no evidence that *S. mansoni* worms present components forming part of the endocytosis of the LDL-receptor complex with the clathrin-coated vesicles. In mammals, LDL binds to receptors on the cell surface followed by internalization of the complex with subsequent proteolytic degradation of LDL [52]. However, it has not been demonstrated that this process occur in *S. mansoni*.

Some authors suggest that human Hsp 70 is involved in the interaction with apo B, present in the LDL, and its transport to the endoplasmic reticulum, as well as being responsible for disrupting clathrin-clathrin interactions, leading to the release of the lipoprotein [18, 53]. Kanehisa (2000) [54] has presented a mechanism in which Hsp 70 in the *S. mansoni* is involved in the disrupting of the clathrin-clathrin interactions and in the transport of lipoprotein into the cell. So far, no protein from the Hsp family was identified as lipoprotein-binding. It is well known that Hsp plays key role in many organisms including schistosomes [55-58]. Among the Hsp family, Hsp 70 is believed to be the most predominantly conserved, acting as intracellular chaperone and in the extracellular transport of immunoregulatory proteins and other molecules [26, 59-61]. Since 1996, it has been showed the presence of Hsp 70 in *S. mansoni* adult worms [62]. In this study, two isoforms of Hsp70 were identified as human plasma LDL-binding

proteins. Hsp 70 is a cytoplasmatic protein capable of interacting with hydrophobic domains of proteins as apo B [18].

There is evidence of mechanisms for transferring lipids and/or proteins of LDL for the adult male worm of *S. mansoni* [47]. The exposure of the worm to human serum caused the appearance on the surface of the worm of LDL binding proteins. [15,16,41]. Whereas previous studies published in the literature, they do not favor the idea that there is an internalization of the LDL in a manner similar to what occurs in humans. However, the transport of lipids in and out of the cells must be done by protein due to the hydrophobic character of those molecules. On other hands, apo B binds to cell surface receptors on the *S. mansoni* [16, 18, 41]. In this way, it can be assumed that even without the internalization of the lipoprotein particle fully, there is a partial internalization of its components. Further studies are required to shed more light on the mechanism of action involved in the acquisition of lipids by the *S. mansoni*.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

This study received support from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). The authors would like to thank Ana Lúcia Oliveira Carvalho from the *Mass Spectrometry and Proteomics Unit* (UEMP), Federal University of Rio de Janeiro, Brazil for her technical assistance.

References

[1] Van Hellemond JJ, Van Balkom BWM, Tielens AGM. Schistosome biology and proteomics: Progress and challenges. Exp Parasitol 2007;117:267-274.

[2] deWalick S, Bexkens ML, van Balkom BWM, Wu Y, Smit CH, Hokke CH, de Groot PG, Heck AJR, Tielens AGM, van Hellemond JJ, De Jong-Brink M. How schitosomes profit from the stress responses they elicit in their hosts. Adv Parasitol 1995;35: 177-256.

[3] World Health Organization (WHO). Schistosomiasis 2013;115:March.

[4] Gryseels B, Polman K, Clerinx J, Kestens L. Human schistosomiasis. Lanc 2006;368: 1106-1118.

[5] Castro-Borges W, Simpson DM, Dowle A, Curwen RS, Thomas-Oates J, Beynon RJ, Wilson RA. Abundance of tegument surface proteins in the human blood fluke *Schistosoma mansoni* determined by QconCAT proteomics. J Prot 2011;74: 1519-1533.

[6] Berriman M et.al. 2009. The genome of the blood fluke *Schistosoma mansoni*. Nat 2009;460:352-360.

[7] Cheng GF, Lin JJ, Feng XG, Fu ZQ, Jin YM., Yuan C X, Zhou YC, Cai YM. Proteomic analysis of differentially expressed proteins between the male and female worm of *Schistosoma japonicum* after pairing. Prot 2005;5: 511-521.

[8] Abath FG, Werkhauser RC. The tegument of *Schistosoma mansoni*: functional and immunological features. Parasite Immunol 1996; 18:15-20.

[9] Mulvenna J, Moertel L, Jones MK, Nawaratna S, Lovas EM, Gobert GN, Colgrave M, Jones A, Loukas A, McManus DP. Exposed proteins of the *Schistosoma japonicum* tegument. Int J Parasitol 2010;40(5): 543-554.

[10] Schramm G, Haas H. Th2 immune response against *Schistosoma mansoni* infection. *Microb Infect* 2010;12:881-888.

[11] Meyer F, Meyer H, Bueding E. Lipid metabolism in the parasitic and free-living flat worms, *Schistosoma mansoni* and *Dugesia dorotocephala*. Biochim Biophys Acta 1970;210: 257-266.

[12] Rumjanek FD, Pereira MAC, Silveira AMV. The interaction of human serum with the surface membrane of Schistosomula of *Schistosoma mansoni*. Mol Biochem Parasitol 1985;14:63-73.

[13] Calvo D, Gomez-Coronado D, Lasuncion MA, Vega MA. CLA-1 is an 85-kD plasma membrane glycoprotein that acts as a high-affinity receptor for both native (HDL, LDL, and VLDL) and modified (OxLDL and AcLDL) lipoproteins. Arterioscler Thromb Vasc Biol 1997;17: 2341-2349.

[14] Tempone AJ, Bianconi ML, Rumjanek FD. The interaction of human LDL with the tegument of adult *Schistosoma mansoni*. *Mol Cell Biochem 1997;*177:139-144.

[15] Rumjanek FD, Mclaren DJ, Smithers SR. Serum-induced expression of a surface protein in *Schistosoma mansoni*: a possible receptor for lipid uptake. Mol Biochem Parasitol 1983;9:337-350.

[16] Chiang CP, Caulfield JP. Human lipoprotein binding to schistosomula of *Schistosoma mansoni*. Displacement by poly-anions, parasite antigen masking, and persistence in young larvae. Am J Pathol 1989;135:1015-1024.

[17] Rogers MV, Henkle KJ, Fidge NH, Mitchell GF. Identification of a multispecific lipoprotein receptor in adult *Schistosoma japonicum* by ligand blotting analyses. Mol Biochem Parasitol 1989;35:79–88.

[18] Zhou M, Wu X, Huang L, Ginsberg HN. Apoprotein B100, an Inefficiently Translocated Secretory Protein, Is Bound to the Cytosolic Chaperone, Heat Shock Protein 70. *J Biol Chem* 1995;270 (2): 25220-25224.

[19] Young A. Structural insights into the clathrin coat. Semin Cell Dev Biol 2007;18:448-458.

[20] Brodsky FM, Chen C, Knuehl C, Towler MC, Wakeham DE. Biological basket weaving: formation and function of clathrin-coated vesicles. Annu Rev Cell Dev Biol 2001;17: 517-568.

[21] Lemmon SK. Clathrin uncoating: Auxilin comes to life. Curr Biol 2001;11: 49-5

[22]Brodin L, Low P, Shupliakov O. Sequential steps in clathrin mediated synaptic vesicle endocytosis. Curr Opin Neurobiol 2000;10:312-320.

[23] Walter P, Gilmore R, Blobel G. Protein translocation across the endoplasmic reticulum. Cell 1984;38:5-8.

[24] Walter P, Lingappa VR. Mechanism of protein translocation across the endoplasmic reticulum membrane Annu Rev Cell Biol 1986;2:499-516.

[25] Frydman J, Nimmesgern E, Ohtsusa K, Hartl F U. Folding of nascent polypeptide chains in a high molecular mass assembly with molecular chaperones. Nat 1994;370: 111–117.

[26] Mayer MP, Bukau B. Hsp70 chaperones: cellular functions and molecular mechanism. Cell Mol Life Sci 2005;62(6):670–668.

[27] Holtzman RL, Schechter I. Expression of different forms of the heat-shock factor during the life cycle of the parasitic helminth *Schistosoma mansoni.* Biochim Biophys Acta 1996;1317: 1-4.

[28] Olivier L, Stirewalt MA. An efficient method for exposure of mice to cercarie of *Schistosoma mansoni*. J Parasitol 1952;38: 18-23.

[29] Duvall R H, Dewitt W B. An improved perfusion technique for recovering adults schistosomes from laboratory animals. *Am J Trop Med Hyg* 1967;16: 483.

[30] Lowry O H, Roserbrought NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951;193: 265-275.

[31] Silva SN, Oliveira KF, Brandt CT, Lima VLM. Estudo dos lipídios em jovens portadores de esquistossomose hepatoesplênica submetidos a tratamento cirúrgico**.** Acta Cir Bras 2002;17(4):247-250.

[32] Ramos TMB, Vasconcelos AM, Carvalho VCO, Lima VLM. Alterations in cholesterol, triglyceride and total phospholipid levels in plasma of *Callithrix jacchus*(sagüi) reinfected by *Schistosoma mansoni***.** Rev Soc Bras Med Trop 2004;37: 37-40.

[33] Bennett MW, Caulfield JP. Specific binding of human low-density lipoprotein to the surface of *Schistosoma mansoni* and ingestion by the parasite. Am J Pathol 1991;138: 1173-1182.

[34] Rogers MV. Do parasites express receptors for host lipoproteins? Parasitol Today 1991;7: 117-120.

[35] Bryant C. Organic acid excretion by helminths. Parasitol Today 1993;9 (2): 58-60.

[36] Loukas A, Jones MK, King LT, Brindley PJ, McManus DP. A receptor for Fc on the surface of schistosomes. Infect and Immun 2001;69: 3646-3651.

[37] Jones MK, Gobert GN, Zhang L, Sunderland P, McManus DP. The cytoskeleton and motor proteins of human schistosomes and their roles in surface maintenance and host parasite interactions. BioEssays 2004;26: 752-765.

[38] Gan XX, Shen LY, Wang Y, Ding JZ, Shen HY, Zeng XP, McManus DP, Brindley PJ, Fan J. Recombinant tegumental protein *Schistosoma japonicum* very low density lipoprotein binding protein as a vaccine candidate against *Schistosoma japonicum*. Mem Inst Oswaldo Cruz 2006;101: 9-13.

[39] Xiao S, Xue J, Shen B. Transmission electron microscopic observation on ultrastructural alterations in *Schistosoma japonicum* caused by mefloquine. Parasitol Res 2010;106:1179-1187.

[40] Fan J, Gan X, Yang W, Shen L, Mcmanus DP, Brindley PJ. A *Schistosoma japonicum* very low-density lipoprotein-binding protein. Int J Biochem Cell Biol 2003;35: 1436-1451.

[41] Chiang CP, Caulfield JP. The binding of human low-density lipoproteins to the surface of schistosomula of *Schistosoma mansoni* is inhibited by polyanions and reduces the binding of anti-schistosomal antibodies. Am J Pathol 1989;134: 1007-1017.

[42] Caulfield J, Chiang C, Yacono PW, Smith LA, Golan DE. Low density lipoproteins bound to Schistosoma mansoni do not alter the rapid lateral diffusion or shedding of lipids in the outer surface membrane. J Cell Sci 1991;99: 167-173.

[43] Xu X, Caufield JP. Characterization of human low density lipoprotein binding proteins on the surface of schistosomula of Schistosoma mansoni. Eur J Cell Biol 1992;57:229-235.

[44] Dias Neto E. Projetos Genoma de Parasitas. Biotec Cien Des Rev 1997; 2: 18-21.

[45] Rumjanek FD, Campos EG, Afonso LC. Evidence for the occurrence of LDL receptors in extracts of schistosomula of *Schistosoma mansoni*. Mol Biochem Parasitol 1988;28: 145-152.

[46] Rogers MV, Quilici D, Mitchell GF, Fidge NH. Purification of a putative lipoprotein receptor from *Schistosoma japonicum* adult worms. Mol Biochem Parasitol 1990;41:93-100.

[47] Pereira ASA, Padilha RJR, Lima-Filho JL, Chaves MEC. Scanning electron microscopy of the human low-density lipoprotein interaction with the tegument of *Schistosoma mansoni*. Parasitol Res 2011;109:1395-1402.

[48] Haseeb MA, Eveland K, Fried B. Histochemical lipid studies on *Schistosoma mansoni* adults maintained in situ and in vitro. Int J Parasitol 1984;14: 83-88.

[49] Haseeb MA, Eveland LK, Fried B, Hayat MA. Transmission electron microscopic observations on lipid release in *Schistosoma mansoni* maintained in vitro. Int J Parasitol 1985;15: 49-53.

[50] Haseeb MA, Fried B, Eveland LK. Histochemical and thin-layer chromatographic analyses of neutral lipids in *Schistosomu japonicum* adults and their worm-free incubates. Int J Parasitol 1986;16: 23 l-236.

[51] Haseeb MA, Fried B, Eveland LK. International *Schistosoma mansoni*: female-dependent lipid secretion in males and corresponding changes in lipase activity. Int J Parasitol 1989;19 (7):705-709.

[52] Lehninger ND, Cox MM. Princípios de Bioquímica de Lehninger. 5. ed. Porto Alegre: Artmed; 2011.

[53] Gusarova V, Caplan AJ, Brodsky JL, Fisher EA. Apoprotein B Degradation is promoted by the molecular chaperones hsp90 and hsp70. *J Biol Chem* 2001;276 (27): 24891-24900.

[54] Kanehisa M. Kyoto University Bioinformatics Center. Post-genome Informatics, Oxford University Press. 2000. http://www.genome.jp/dbget-bin/www_bget?pathway:smm04144 access: 17-01-2014.

[55] Maresca B, Carratu L. The biology of the heat shock response in parasite. Parasitol Today 1992;8(8):260–266.

[56] Neumann S, Ziv E, Lantner F, Schechter I. Regulation of HSP70 gene expression during the life cycle of the parasitic helminth *Schistosoma mansoni*. Eur J Biochem 1993; 212(2):589-596.

[57] Smirlis D, Duszenko M, Ruiz AJ, Scoulica E, Bastien P, Fasel N, Soteriadou K. Targeting essential pathways in trypanoso-matids gives insights into protozoan mechanisms of cell death. Parasit Vectors 2010;3:107.

[58] Yang J, Yang L, Lv Z, Wang J, Zhang Q, Zheng H, Wu Z. Molecular cloning and characterization of a HSP70 gene from *Schistosoma japonicum*. Parasitol Res 2012;110:1785-1793.

[59] De Jong-Brink M. How schitosomes profit from the stress responses they elicit in their hosts. Adv Parasitol 1995;35:177-256.

[60] Martinez J, Perez-Serrano J, Bernadina WE, Rincon I, Rodriguez-Caabeiro F. Heat shock protein synthesis over time in infective *Trichinella spiralislarvae* raised in suboptimal culture conditions. J Helminthol 2004;78(3):243–247.

[61] Kimura K, Tanaka N, Nakamura N, Takano S, Ohkuma S. Knockdown of mitochondrial heat shock protein 70 promotes progeria-like phenotypes in *Caenorhabditis elegans*. J Biol Chem 2007;282(8):910-5918.

[62] Levy-Holtzman R, Schechter I. Expression of different forms of the heat-shock factor during the life cycle of the parasitic helminth *Schistosoma mansoni* Biochem Biophys Acta 1996;1317: 1-4.

Figure Captions

Fig. 1. Transmission electron micrographs of *Schistosoma mansoni* adult worms incubated with human serum (HS), anti-LDL antibody and protein-A-gold-complex. Ultrathin sections were exposed to HS diluted 1:10, for 90 min, followed by incubations with chicken anti-LDL antibody (1:1000) for 5 h and protein A conjugated to 20 nm gold particles diluted 1:50 for 30 min. **(A)** Tegument without incubation with LDL (control). **(B)** Label on muscular and syncytial regions. **(C)** Gold particles in and around spine. **(D)** Binding site in vitelline cells of females. Bars = 1 μm; muscular region (mr), syncytial region (rs), spine (sp), vitelline cells (vc).

Fig. 2. 2D electrophoresis gel of *Schistosoma mansoni* adult worm extract and corresponding western blotting. **(A**) 2D electrophoresis using 400 μg of proteins, 13 cm pH 3–10 NL strip, 10 % SDS-PAGE and coomassie staining. **(B)** Western blotting on PVDF membrane, incubated with anti-LDL and anti-chicken IgY (IgG) peroxidase conjugate, revealed using TMB substrate. The numbers correspond to those in Supporting Information, Table 1

Figure 1
Click here to download high resolution image

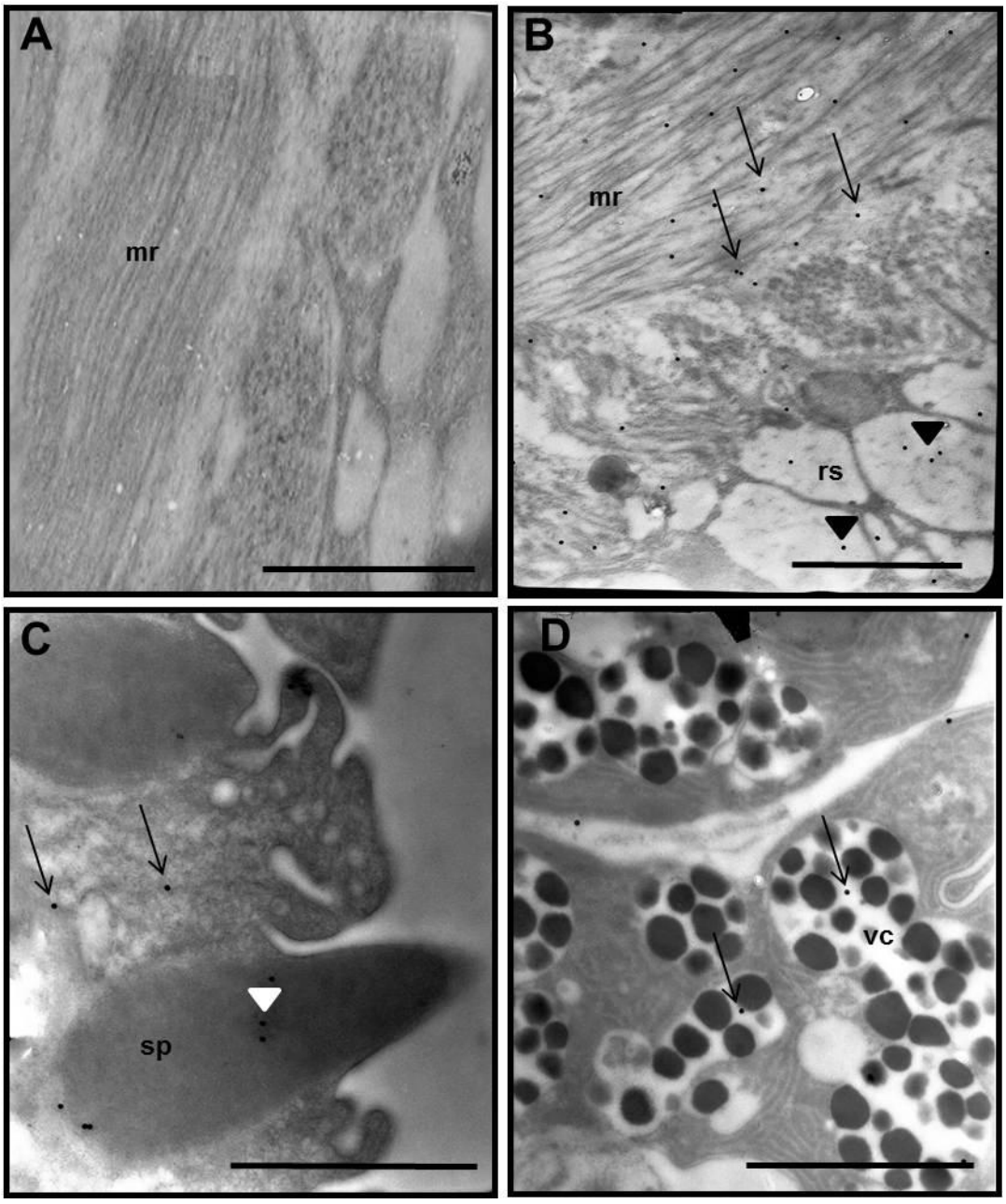

Figure 2
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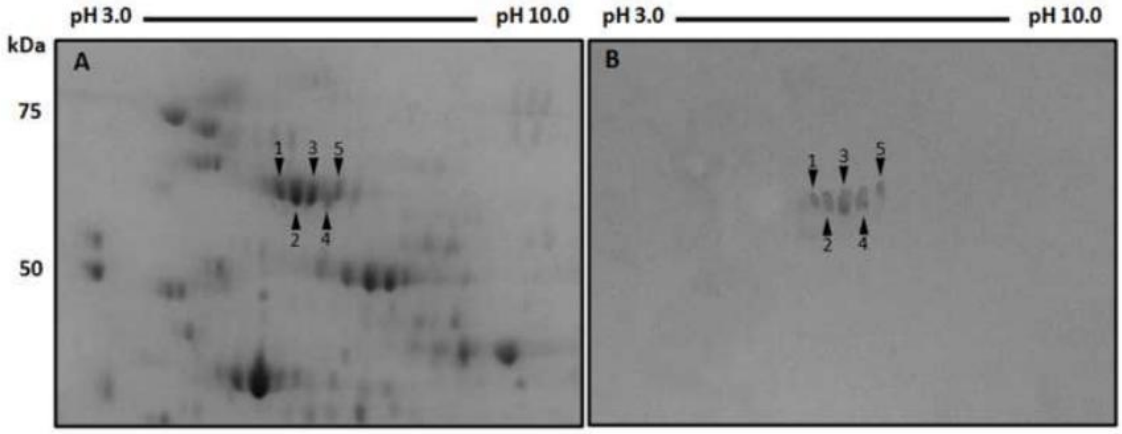

Table 1

Table 1: Proteins involved in the interaction of the LDL with the *Schistosoma mansoni*.

Sample	Accession number (NCBI BLAST)	Protein	Peptides Matches	Number on unique peptides identified	Decoy (%) Identity	Decoy (%) Homology	Nominal mass (M_r)	Experimental mass	Calculated pI	Experimental pI	SCORE	Coverage
1	gi|353229993	heat shock protein 70 [*Schistosoma mansoni*]	42(7)	9	0	3	70215	73030	5.42	5.84	1098	46%
2	gi|353229993	heat shock protein 70 [*Schistosoma mansoni*]	47(14)	8	0	0	70215	72433	5.42	5.93	1269	44%
3	gi|353229993	heat shock protein 70 [*Schistosoma mansoni*]	49(8)	10	0	2	70215	71645	5.42	6.04	1113	46%
4	gi|353229993	heat shock protein 70 [*Schistosoma mansoni*]	22(4)	5	0	1	70215	72831	5.42	6.19	655	32%
5	gi|256090832	heat shock protein 70 [*Schistosoma mansoni*]	28(3)	14	0	0	71595	72038	6.09	6.30	831	35%

***Graphical abstract**

Isoforms of Hsp70-binding human LDL in adult worms of *Schistosoma mansoni*

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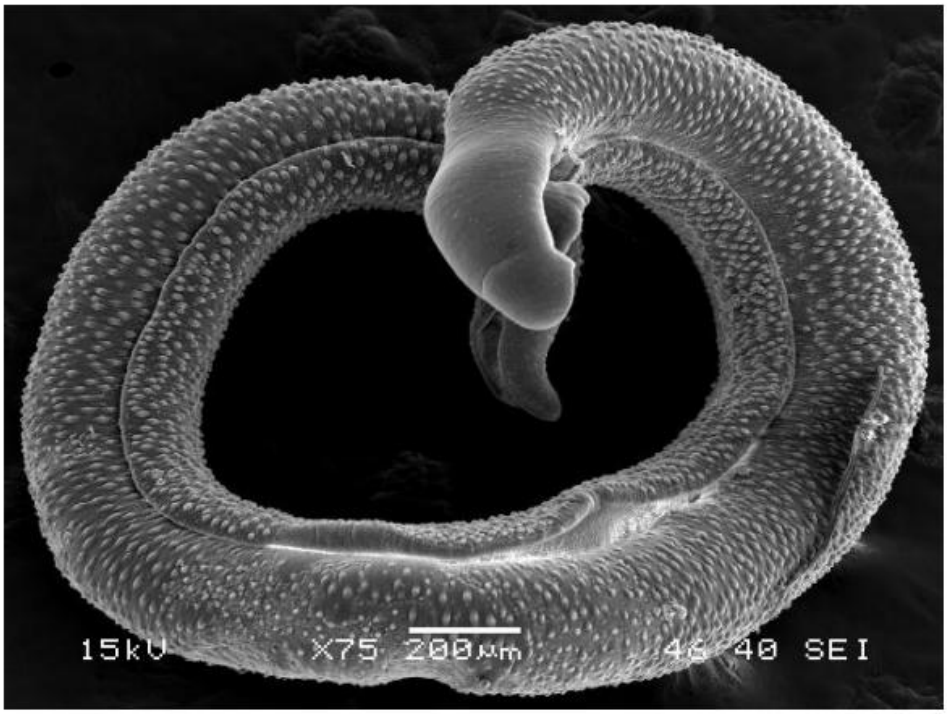
Schistosoma mansoni does not synthesize long fatty acids, but acquire from the host's organism. Human LDL can be a source of lipids for the Schistosoma.



8. CONSIDERAÇÕES FINAIS

- Os valores morfométricos encontrados para cercarias e vermes adultos da cepa SLM do *S. mansoni* foram menores do que outras cepas descritas na literatura;
- Lipoproteínas de baixa densidade (LDL) se ligam ao tegumento do verme adulto macho de *Schistosoma mansoni*, ocorrendo maior interação das partículas de LDL na região dorsal mediana do que na região posterior do verme;
- Na região anterior aparentemente não há ligação de lipoproteínas, assim como no canal ginecófaro;
- Partículas de ouro foram observadas no tegumento, na região muscular e nas espículas de vermes machos e nas células vitelínicas das fêmeas;
- Por eletroforese bidimensional e immunoblotting foram identificados pelo anticorpo anti-LDL 5 "spots", com pontos isoelétricos (PIS) entre 5.8 e 6.3, e massas moleculares (MM) entre 71 e 73 kDa;
- A análise dos "spots" por LC MS/MS identificou duas diferentes formas da proteína de choque térmico 70 (Heat schock protein 70 – Hsp 70) com alta cobertura e distribuição uniforme de aminoácidos;
- O verme adulto de *S. mansoni* apresenta proteínas ligantes de LDL, supondo-se que mesmo sem uma internalização total da partícula de Lipoproteína exista uma internalização parcial de seus componentes e a Hsp 70 pode funcionar no transporte da LDL ou de fragmentos desta no interior do verme.

9. ANEXOS

9.1 Aprovação da Comissão de Ética em Experimentação Animal (CEEA) da UFPE

Universidade Federal de Pernambuco
Centro de Ciências Biológicas
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Ofício nº 43/07 Recife, 21 de maio de 2007

Da Comissão de Ética em Experimentação Animal (CEEA) da UFPE
Para: **Prof. José Luiz de Lima Filho**
Laboratório de Imunopatologia Keizo Asami/LIKA - UFPE
Processo nº 016275/2006-81

Os membros da Comissão de Ética em Experimentação Animal do Centro de Ciências Biológicas da Universidade Federal de Pernambuco (CEEA-UFPE) avaliaram a resposta de V. Sa. referente ao primeiro parecer da CEEA sobre o projeto de pesquisa intitulado "**Estudo de classes de proteínas do *Schistosoma mansoni* por análise proteômica**".

Concluímos que os procedimentos descritos para a utilização experimental dos animais encontram-se de acordo com as normas sugeridas pelo Colégio Brasileiro para Experimentação Animal e com as normas internacionais estabelecidas pelo National Institute of Health Guide for Care and Use of Laboratory Animals as quais são adotadas como critérios de avaliação e julgamento pela CEEA-UFPE.

Encontra-se de acordo com as normas vigentes no Brasil, especialmente a Lei 9.605 – art. 32 e Decreto 3.179-art 17, de 21/09/1999, que trata da questão do uso de animais para fins científicos.

Diante do exposto, emitimos **parecer favorável** aos protocolos experimentais realizados.

Atenciosamente,
Profª. Silene Carneiro do Nascimento
Presidente CEEA

CCB: Integrar para desenvolver