Scientific Research Publishing

Open Journal of Animal Sciences, 2019, 9, 173-182
http://www.scirp.org/journal/ojas
ISSN Online: 2161-7627
ISSN Print: 2161-7597

Schistosoma mansoni Infection in *Holochilus sciureus* Shows Sex-Related Differences in Parasitological Patterns

Guilherme Silva Miranda[1,2*], João Gustavo Mendes Rodrigues[3], Maria Gabriela Sampaio Lira[4], Ranielly Araújo Nogueira[4], Gleycka Cristine Carvalho Gomes[4], Nêuton Silva-Souza[4]

[1]Department of Parasitology, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, Brazil
[2]Teaching Department, Federal Institute of Maranhão, São Raimundo das Mangabeiras, Brazil
[3]Department of Pathology, Centre for Biological and Health Sciences, Federal University of Maranhão, São Luís, Brazil
[4]Department of Chemistry and Biology, Laboratory of Human Parasitology, State University of Maranhão, São Luís, Brazil
Email: *mirandagsbio@igmail.com

How to cite this paper: Miranda, G.S., Rodrigues, J.G.M., Lira, M.G.S., Nogueira, R.A., Gomes, G.C.C. and Silva-Souza, N. (2019) *Schistosoma mansoni* Infection in *Holochilus sciureus* Shows Sex-Related Differences in Parasitological Patterns. *Open Journal of Animal Sciences*, **9**, 173-182.
https://doi.org/10.4236/ojas.2019.92015

Received: January 15, 2019
Accepted: March 26, 2019
Published: March 29, 2019

Abstract

Due to the different parasitological patterns found between sexes in human populations and in different biological models during *Schistosoma mansoni* infection, we proposed to investigate such differences using *Holochilus sciureus* rodent, besides confirming whether this rodent is suitable for experimental infections. In this sense, males and females of *H. sciureus* were infected with 200 cercariae from a human strain of *S. mansoni*. The number of eggs per gram of feces (epg) and the worms were quantified, besides histopathological analysis. Thus, it was shown that females have fewer epg, as well as a longer prepatent period. On the other hand, males had a lower recovery rate of adult worms. The histopathological analysis did not show differences between the sexes. Thus, we suggest that infection in *H. sciureus* females provides a favorable environment for the development of adult worms, despite impairing the parasite fecundity. In addition, *H. sciureus* may be an excellent biological model for *S. mansoni* experimental infections.

Keywords

Biological Model, Females, Males, Schistosomiasis, Wild Reservoir

1. Introduction

Schistosomiasis is a serious endemic parasitic disease caused by trematodes of the genus *Schistosoma*. It is distributed in 78 countries around the world and affects 200 - 250 million people [1] [2] [3]. In Brazil, prevalence data from this

disease show around 2.5 and 8 million of infected people [4] [5]. One of the peculiarities presented in the epidemiology of schistosomiasis in Brazil is the presence of wild rodents with semi-aquatic habits (mainly *Holochilus sciureus* and *Nectomys squamipes*) naturally parasitized by *S. mansoni* [6] [7].

Studies suggest that these rodents are able to maintain the parasite's life cycle in the absence of the humans [7] [8]. Due to the fact that some murine models used in experimental infections have limitations in mimicking human disease [9], these wild reservoirs are suggested as suitable biological models [10].

Interestingly when the rodent *Holochilus brasiliensis* was used in *S. mansoni* experimental infections with a wild strain, females showed less severe hepatic lesions, when compared with male rodents [11]. In this sense, several studies with human population and murine models describe that there is a clear relationship between sex and susceptibility/resistance in relation to different infections, and there is a general consensus that males are more susceptible than females regarding viral, bacterial, parasitic and fungal infections [12].

In order to elucidate the relationship between sex and the susceptibility/resistance to *S. mansoni* infection, the use of *H. sciureus* as an experimental model is valuable to the understanding of differences also found in human populations infected by this parasite. For this purpose, characterisation of parasitological and histopathological features during *S. mansoni* experimental infections in male and female *H. sciureus* will be described hereafter.

2. Material and Methods

2.1. Animals and Experimental Infection

H. sciureus with 3 month-old were obtained from the Central Animal Facility of State University of Maranhão (UEMA), where the specimens have been reared for at least 5 generations. The lineage was started in captivity from specimens collected in the municipality of São Bento, Maranhão State, with SISBIO authorization (No. 40025/1 and registry 543545).

For the experimental infection, a human strain of *S. mansoni* from São Luís city, Maranhão State was used. It has been maintained in successive passages through *Biomphalaria glabrata* mollusks and Balb/c mice at the Laboratory of Human Parasitology, Department of Chemistry and Biology (UEMA).

The cercariae were harvested from infected *B. glabrata*, washed, counted, and injected subcutaneously into each rodent (200 per rodent) as previously described by Pellegrino and Macedo [13]. In the same experimental replicate, all groups were injected with the same cercaria suspension. Mortality rate during the infection period, which lasted nine weeks, was measured. For parasitological analysis, a total of 8 animals of each sex were used in each of the three independent experiments. For histopathological analysis, this number was reduced to 2 animals of each sex, also in triplicate. Following manipulation, animals were kept individually in polypropylene cages in rooms at 28˚C ± 2˚C and about 70% relative humidity. Water and food were given *ad libitum*.

The research was approved by the Council of Ethics and Animal Experimentation of the State University of Maranhão (No. 04/2014).

2.2. *S. mansoni* Parasite Burden

After 30 days post-infection, once a week, until the time of euthanasia, the feces of each animal were collected for preparation of three Kato-Katz slides, in order to quantify the number of eggs [14].

The animals were euthanised with overdose of intraperitoneal injection with ketamine 5% and xylazine hydrochloride 2% [15]. The circulatory system of each rodent from the experimental group was perfused with an isotonic saline solution containing 1000 IU/L of heparin after eight weeks of *S. mansoni* infection, as previously described by Pellegrino and Siqueira [16]. Worms recovered from each infected rodent were counted with the aid of a stereomicroscope. Male and female worms were morphologically identified and counted [17].

2.3. Histopathologic Evaluation

The right lobes from livers from not perfused infected *H. sciureus* were readily immersed in 4% formaldehyde prepared in phosphate-buffered saline (PBS) and incubated at room temperature for 18 h. The tissue samples were then washed, transferred into 70% ethanol and embedded in paraffin. Five-micrometer-thick sections were stained with hematoxylin and eosin (H&E) to evaluate the inflammatory infiltration [18]. The severity of these lesions was classified qualitatively as mild, moderate and intense, according to the characteristics proposed by Silva-Souza and Vasconcelos [11].

2.4. Statistical Analysis

All the data were initially analyzed with the Shapiro-Wilk test. To compare the mean values, Student's t-test was carried out. The results were considered significant when the p-value was <0.05. The data were analyzed, and the graphs created using GraphPad-Prism 6 software (Prism Software, Irvine, California, USA).

3. Results

Our data showed that there was no mortality of the infected animals. In addition, approximately 90% of males were eliminating eggs in feces, compared to 80% of females. It was also observed that male rodents had a shorter prepatent period, from 43 to 45 days post-infection (6th week), than females (50 to 53 days post-infection/7th week) (**Figure 1(A)**).

It was observed that female rodents eliminated on average 47.63 ± 41.37 epg while males eliminated 80.25 ± 65.86 epg. Even though these data was not statistically significant, when analyzed on a weekly basis, it was verified that from the 7th week post-infection, females showed a lower elimination rate of *S. mansoni* eggs when compared to males rodents (**Figure 1(A)**).

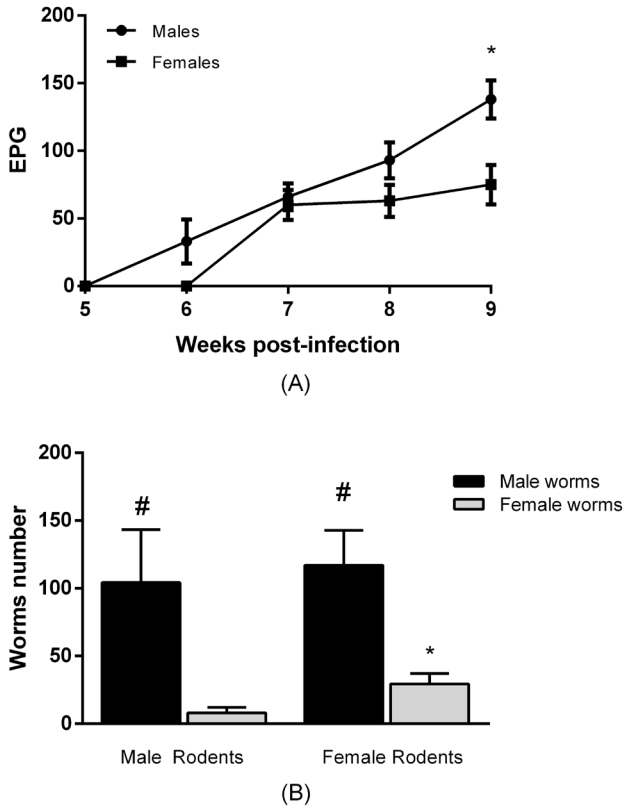

Figure 1. Comparative description of the parasitic burden between male and female *H. sciureus* experimentally infected with human *S. mansoni* strain. (A) Quantification of eggs in feces during nine weeks post-infection. (B) Number of worms (males and females) collected from the porta-hepatic system of male and female rodents. The means (with Std. Error) were compared by Student's t-test. Symbols: (#) statistically different when comparing the same group and (*) between different groups (males *vs.* females).

Contradictorily, we demonstrated that the number of worms (males and females) was lower in male than in female rodents. In general, female worms were found in fewer numbers than male worms, for both rodents sexes (**Figure 1(B)**). The worm recovery rate was 56.12% for male and 73.18% for female rodents.

Although we demonstrated differences in the parasitological profile between males and females, we found similar patterns of hepatic lesions (mild to moderate), with a large amount of granulomas distributed throughout the tissue, with intense inflammatory infiltrate (mainly by the presence of eosinophils) and disorganized deposition of extracellular matrix (**Figure 2(A)** and **Figure 2(B)**). Occasionally adult parasites were also found (**Figure 2(C)** and **Figure 2(D)**). Next we demonstrate the variety of hepatic lesions found in *H. sciureus*, regardless of sex.

4. Discussion

In experimental studies using *S. mansoni* infection, the choice of biological model and parasite strain to be used must be well through, depending on the aim to be achieved by the research, since there may be differences in the rate of

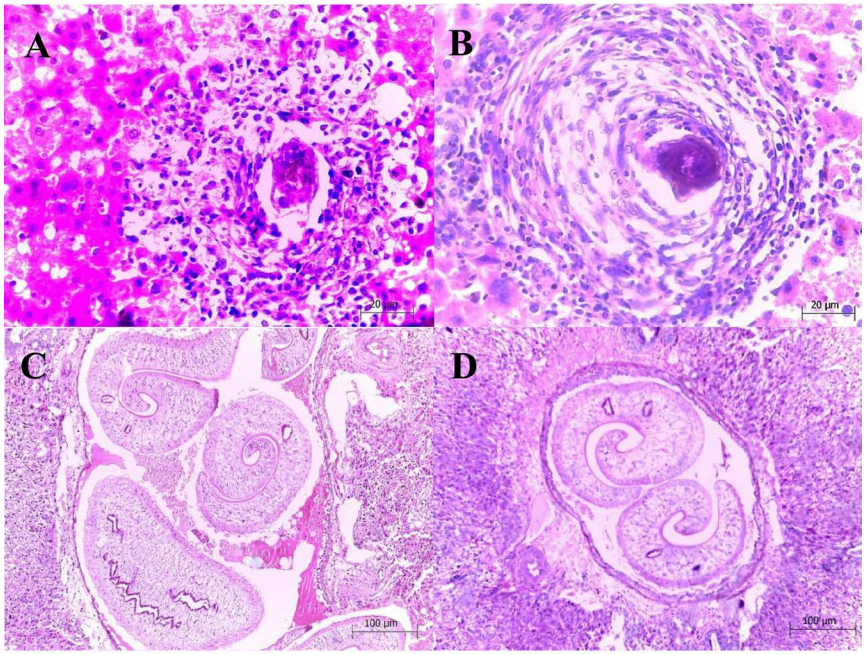

Figure 2. Photomicrography of the hepatic parenchyma of *H. sciureus* experimentally infected with human *S. mansoni* strain. (A) and (B) Presence of a typical granulomatous reaction around the *S. mansoni* egg (400x). (C) and (D) Presence of adult parasites in intrahepatic branches (100×). Tissue fragments were stained with eosin and haematoxylin.

recovery and fecundity of adult worms and consequently different immunopathological profiles during infection [19] [20] [21] [22] [23]. However, few studies also analyze the animal model sex as a determinant factor in the evolution of *S. mansoni* infection.

In human populations, studies report that men present a higher prevalence for *S. mansoni* than women, with the justification that social behavior favors men to have a greater exposure to environments with a higher risk of infection due to leisure or occupational activities, such as bathing, fishing and sports [24] [25]. Nevertheless, social behavior is not sufficient to explain gender-related differences in infection, because women in several endemic regions are involved in similarly risky activities such as doing laundry in rivers [26].

We also emphasize that a lower prevalence of schistosomiasis in women may be due to a lower number of eggs eliminated in feces, as observed in *H. sciureus* females in our study, which would decrease the sensitivity of diagnostic tests. However, this has yet to be further investigated.

Studies in experimental models have shown that females of CBA/J mice experimentally infected with *S. mansoni* from Puerto Rico strain showed a higher susceptibility to infection, with a higher rate of recovery of adult worms than male mice, but hepatic lesions were similar for both sexes [27]. Although this study uses a different experimental model from ours, these data together may suggest that not only the infection with *S. mansoni* strains from Brazil leads to sex-related differences in parasitological patterns.

On the other hand, it is necessary to consider the changes in pathological features induced by different strains from the same region. Silva-Souza and Vasconcelos [11], using *H. brasiliensis* as an experimental model of *S. mansoni* infection (from Brazil wild strain), found differences in hepatic pathology between sexes, in which females presented less severe lesions. This differs from our data from a human strain from Brazil.

Additionally, when analyzing data obtained with experimental infections with *S. mansoni* from the Egypt strain in different experimental models of both sexes, it was possible to verify that in two experiments with female hamsters (*Mesocricetus auratus*), a rate of 52.31% worms was recovered (males and females) [28]. Opposite to this, in male Balb/c and C57 mice, a worm recovery rate of 5.4% and 25% was observed, respectively [29]. Still, when a *S. mansoni* from the Kenya strain was used to infect three female baboons (*Papio anubis*), one of the experiments demonstrated a high success rate in the recovery of adult worms (70.5%) [30], which was expected as they are genetically more similar to humans.

Although it is not possible to certainly imply the influence of sex in these cases, since other studies in parallel with the opposite sex were not performed, it is clearly noticeable the difference in susceptibility of *S. mansoni* in different vertebrate hosts. In this sense, we call attention to the rate of recovery of worms from *H. sciureus*, which, regardless of sex, in our study was 64.65% (with a higher proportion of male worms), demonstrating that this rodent can be considered an excellent reservoir and experimental model of *S. mansoni*. According to Boissier and Moné [31] the more permissive host has a higher rate of recovery of worms, in addition to showing an increased proportion of male worms.

Few studies were performed regarding the number of *S. mansoni* eggs eliminated in feces in different experimental models and analyzed by sex. However, it has already been demonstrated that the experimental infection by *S. mansoni* (BH human strain) in two other species of wild reservoirs found in Brazil (*N. rattus* and *N. squamipes*) of both sexes, was found a prepatent period which ranged from 40 to 42 days, and an amount of eggs per gram of feces ranging from 0 - 264 [32]. These results were similar to the parasitological profile found for *H. sciureus* in our study, mainly for males, suggesting that *S. mansoni* infection in different species of wild rodents, considered reservoirs of this parasite in Brazil, may present a similar parasitological profile.

The differences in the parasitological aspects found in our study may be related to the fact that females may present different immunological responses due to the influence of female sex hormones. These hormones are hypothesized to underlie sex differences in infection via effects on disease-resistance genes, immunity, and behavior [12] [33].

Generally, in human populations, the percentage of T lymphocytes within the total lymphocyte population in men is lower when compared to women [34], probably due to the increased testosterone concentrations, which enhance the activity of T cell apoptosis [35]. On the other hand, the estrogens increase bone

marrow progenitor B cells numbers in mice by protecting the progenitor cells from apoptosis [36] [37]. Therefore the different immunopathological processes during *S. mansoni* infection in different experimental models and parasite strains may also be due to immunological differences between the sexes that influence the functioning of immune cells.

Thus, in summary, we demonstrated that *H. sciureus* can be considered an excellent biological model in experimental infections of *S. mansoni* due to the low mortality of these rodents and the great recovery of adult worms. Nonetheless, complementary studies with a longer infection (chronic phase) and with egg counts in different tissues, in our experimental model, still need to be performed. In addition, it has been shown that sex may influence parasitological patterns during infection, and that these differences may be related to sex hormones. In this sense, we suggest that further studies with *S. mansoni* experimental infection should not be based solely on one sex of the biological model and that the role of sex hormones should be better investigated.

Acknowledgements

We thank the Fundação de Amparo à Pesquisa e ao Desenvolvimento Científico e Tecnológico do Maranhão, FAPEMA and the Universidade Estadual do Maranhão, for financial support. We also thank Prof. Dra. Débora Martins Silva Santos (Department of Chemistry and Biology, State University of Maranhão, São Luís, Brazil) for assistance in the preparation of tissue fragments for histological slides.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Oliveira, S.C., Fonseca, C.T., Cardoso, F.C., Farias, L.P. and Leite, L.C. (2008) Recent Advances in Vaccine Research against Schistosomiasis in Brazil. *Acta Tropica*, **108**, 256-262. https://doi.org/10.1016/j.actatropica.2008.05.023

[2] Toledo, R. and Fried, B. (2014) Digenetic Trematodes, Advances in Experimental Medicine and Biology. Springer-Verlag, New York. https://doi.org/10.1007/978-1-4939-0915-5

[3] GBD 2016 DALYs and HALE Collaborators. (2017) Global, Regional, and National Disability-Adjusted Life Years (DALYs) for 333 Diseases and Injuries and Healthy Life Expectancy (HALE) for 195 Countries and Territories, 1990-2016: A Systematic Analysis for the Global Burden of Disease Study 2016. *The Lancet*, **390**, 1260-1344. https://doi.org/10.1016/S0140-6736(17)32130-X

[4] Katz, N. and Peixoto, S.V. (2000) Critical Analysis of the Estimated Number of Schistosomiasis Mansoni Carriers in Brazil. *Journal of the Brazilian Society of Tropical Medicine*, **33**, 303-308. https://doi.org/10.1590/S0037-86822000000300009

[5] Rollemberg, C.V.V., Santos, C.M.B., Silva, M.M.B.L., Souza, A.M.B., Silva, A.M., Almeida, J.A.P., Almeida, R.P. and Ribeiro-Jesus, A. (2011) Epidemiological Cha-

racteristics and Geographical Distribution of Schistosomiasis and Geohelminths, in the State of Sergipe, Sccording to Data from the Schistosomiasis Control Program in Sergipe. *Journal of the Brazilian Society of Tropical Medicine*, **44**, 91-96. https://doi.org/10.1590/S0037-86822011000100020

[6] Rey, L. (1993) Non-Human Vertebrate Hosts of *Schistosoma mansoni* and Schistosomiasis Transmission in Brazil. *Research and Reviews in Parasitology*, **53**, 13-25.

[7] Miranda, G.S., Miranda, B.S., Rodrigues, J.G.M., Lira, M.G.S., Nogueira, R.A., Viegas-Melo, D. and Silva-Souza, N. (2017) Research Note. The Wild Water-Rats and Their Relevance in the Context of Schistosomiasis mansoni in Brazil: What We Know and Recommendations for Further Research. *Helminthologia*, **54**, 165-169. https://doi.org/10.1515/helm-2017-0013

[8] Kawazoe, U. and Pinto, A.C.M. (1983) Epidemiological Importance of Some Wild Animals in Schistosomiasis mansoni. *Journal of Public Health*, **17**, 345-366.

[9] Wilson, R.A., Li, X.H. and Castro-Borges, W. (2016) Do Schistosome Vaccine Trials in Mice Have an Intrinsic Flaw that Generates Spurious Protection Data? *Parasites & Vectors*, **9**, 89. https://doi.org/10.1186/s13071-016-1369-9

[10] Bastos, O.D.C., Sadigursky, M., Nascimento, M.D.D.S.B., Brazil, R.P. and Holanda, J.C.D. (1984) *Holochilus brasiliensis nanus* Thomas, 1897: as an Experimental Model for Filariasis, Leishmaniasis and Schistosomiasis. *Journal of the Institute of Tropical Medicine of São Paulo*, **26**, 307-315. https://doi.org/10.1590/S0036-46651984000600004

[11] Silva-Souza, N. and Vasconcelos, S.D. (2005) Histopathology of *Holochilus brasiliensis* (Rodentia: Cricetidae) Infected with *Schistosoma mansoni* (Schistosomatida: Schistosomatidae). *Journal of Tropical Pathology*, **34**, 145-150. https://doi.org/10.5216/rpt.v34i2.1920

[12] Klein, S.L. (2000) The Effects of Hormones on Sex Differences in Infection: From Genes to Behavior. *Neuroscience Biobehavioral Reviews*, **24**, 627-638. https://doi.org/10.1016/S0149-7634(00)00027-0

[13] Pellegrino, J. and Macedo, D.G. (1955) A Simplified Method for Concentration of Cercariae. *Journal of Parasitology*, **41**, 306-309. https://doi.org/10.2307/3274230

[14] Katz, N., Chaves, A. and Pellegrino, J. (1972) A Simple Device for Quantitative Stool Thick-Smear Technique in *Schistosomiasis mansoni*. *Journal of the Institute of Tropical Medicine of São Paulo*, **14**, 397-400.

[15] Andrade, A., Pinto, S.C. and Oliveira, R.S. (2002) Laboratory Animals: Creation and Experimentation. Fiocruz, Rio de Janeiro, 388.

[16] Pellegrino, J. and Siqueira, A.F.A. (1956) Perfusion Technic for Recovery of *Schistosoma mansoni* from Experimentally Infected Guinea Pigs. *Brazilian Journal of Malariology and Tropical Diseases*, **8**, 589-597.

[17] Neves, R.H., Oliveira, A.S., Machado-Silva, J.R., Coutinho, E.M., Lenzi, H.L. and Gomes, D.C. (2002) Phenotypic Characterization of *Schistosoma mansoni* Adult Worms Recovered from Undernourished Mice: A Morphometric Study Focusing on the Reproductive System. *Journal of the Brazilian Society of Tropical Medicine*, **35**, 405-407. https://doi.org/10.1590/S0037-86822002000400019

[18] Luna, L.G. (1968) Manual of Histological Staining Methods of the Armed Force Institute of Pathology. 3rd Edition, McGraw-Hill, New York, 258.

[19] Jones, J.T., Breeze, P. and Kusel, J.R. (1989) Schistosome Fecundity: Influence of Host Genotype and Intensity of Infection. *International Journal for Parasitology*, **19**, 769-777. https://doi.org/10.1016/0020-7519(89)90065-9

[20] Cheever, A.W., Macedonia, J.G., Mosimann, J.E. and Cheever, E.A. (1994) Kinetics of Egg Production and Egg Excretion by *Schistosoma mansoni* and *S. japonicum* in Mice Infected with a Single Pair of Worms. *The American Journal of Tropical Medicine and Hygiene*, **50**, 281-295. https://doi.org/10.4269/ajtmh.1994.50.281

[21] Incani, R.N., Morales, G. and Cesari, I.M. (2001) Parasite and Vertebrate Host Genetic Heterogeneity Determine the Outcome of Infection by *Schistosoma mansoni*. *Parasitology Research*, **87**, 131-137. https://doi.org/10.1007/PL00008565

[22] Yoshioka, L., Zanotti-Magalhães, E.M., Magalhães, L.A. and Linhares, A.X. (2002) *Schistosoma mansoni*: A Study of Pathogenesis of Santa Rosa Strain (Campinas, SP, Brasil) in Mice. *Journal of the Brazilian Society of Tropical Medicine*, **35**, 203-207. https://doi.org/10.1590/S0037-86822002000300001

[23] Alves, C.C., Araujo, N., Cassali, G.D. and Fonseca, C.T. (2016) Parasitological, Pathological, and Immunological Parameters Associated with *Schistosoma mansoni* Infection and Reinfection in BALB/c and C57BL/6 Mice. *Journal of Parasitology*, **102**, 336-341. https://doi.org/10.1645/14-664

[24] Guimarães, I.C.S. and Tavares-Neto, J. (2006) Urban Transmission of Schistosomiasis in Children from a Neighborhood of Salvador, Bahia. *Journal of the Brazilian Society of Tropical Medicine*, **39**, 451-455. https://doi.org/10.1590/S0037-86822006000500006

[25] Palmeira, D.C.C., Carvalho, A.G., Rodrigues, K. and Couto, J.L.A. (2010) Prevalence of *Schistosoma mansoni* Infection in Two Municipalities of the State of Alagoas, Brazil. *Journal of the Brazilian Society of Tropical Medicine*, **43**, 313-317. https://doi.org/10.1590/S0037-86822010000300020

[26] Fulford, A.J.C., Ouma, J.H., Kariuki, H.C., Thiongo, F.W., Klumpp, R., Kloos, H., Sturrock, R.F. and Butterworth, A.E. (1996) Water Contact Observations in Kenyan Communities Endemic for Schistosomiasis: Methodology and Patterns of Behaviour. *Parasitology*, **113**, 223-241. https://doi.org/10.1017/S0031182000082007

[27] Eloi-Santos, S., Olsen, N.J., Correa-Oliveira, R. and Colley, D.G. (1992) *Schistosoma mansoni*: Mortality, Pathophysiology, and Susceptibility Differences in Male and Female Mice. Experimental Parasitology, 75, 168-175. https://doi.org/10.1016/0014-4894(92)90176-B

[28] El Ridi, R., Tallima, H., Salah, M., Aboueldahab, M., Fahmy, O.M., Al-Halbosiy, M.F. and Mahmoud, S.S. (2012) Efficacy and Mechanism of Action of Arachidonic Acid in the Treatment of Hamsters Infected with *Schistosoma mansoni* or *Schistosoma haematobium*. *International Journal of Antimicrobial Agents*, **39**, 232-239. https://doi.org/10.1016/j.ijantimicag.2011.08.019

[29] Dajem, S.M.B., Mostafa, O.M. and El-Said, F.G. (2008) Susceptibility of Two Strains of Mice to the Infection with *Schistosoma mansoni*: Parasitological and Biochemical Studies. *Parasitology Research*, **103**, 1059-1063. https://doi.org/10.1007/s00436-008-1092-3

[30] Yole, D.S., Pemberton, R., Reid, G.D. and Wilson, R.A. (1996) Protective Immunity to *Schistosoma mansoni* Induced in the Olive Baboon *Papio anubis* by the Irradiated Cercaria Vaccine. *Parasitology*, **112**, 37-46. https://doi.org/10.1017/S0031182000065057

[31] Boissier, J. and Moné, H. (2001) Relationship between Worm Burden and Male Proportion in *Schistosoma mansoni* Experimentally Infected Rodents and Primates. A Meta-Analytical Approach. *International Journal for Parasitology*, **3**, 1597-1599. https://doi.org/10.1016/S0020-7519(01)00277-6

[32] Ribeiro, A.C., Maldonado Jr., A., D'Andrea, P.S., Vieira, G.O. and Rey, L. (1998)

Susceptibility of *Nectomys rattus* (Pelzen, 1883) to Experimental Infection with *Schistosoma mansoni* (Sambon, 1907): A Potential Reservoir in Brazil. *Memórias do Instituto Oswaldo Cruz*, **93**, 295-299. https://doi.org/10.1590/S0074-02761998000700058

[33] Klein, S.L., Jedlicka, A. and Pekosz, A. (2010) The Xs and Y of Immune Responses to Viral Vaccines. *Lancet Infectious Diseases*, **10**, 338-349. https://doi.org/10.1016/S1473-3099(10)70049-9

[34] Bouman, A., Schipper, M., Heineman, M.J. and Faas, M.M. (2004) Gender Difference in the Non-Specific and Specific Immune Response in Humans. *American Journal of Reproductive Immunology*, **52**, 19-26. https://doi.org/10.1111/j.1600-0897.2004.00177.x

[35] McMurray, R.W., Suwannaroj, S., Ndebele, K. and Jenkins, J.K. (2001) Differential Effects of Sex Steroids on T and B Cells: Modulation of Cell Cycle Phase Distribution, Apoptosis and Bcl-2 Protein Levels. *Pathobiology*, **69**, 44-58. https://doi.org/10.1159/000048757

[36] Medina, K.L., Strasser, A. and Kincade, P.W. (2000) Estrogen Influences the Differentiation, Proliferation, and Survival of Early B-Lineage Precursors. *Blood*, **95**, 2059-2067.

[37] Grimaldi, C.M., Cleary, J., Dagtas, A.S., Moussai, D. and Diamond, B. (2002) Estrogen Alters Thresholds for B Cells Apoptosis and Activation. *Journal of Clinical Investigation*, **109**, 1625-1633. https://doi.org/10.1172/JCI0214873