ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 10, Issue, 06, pp.70818-70823, June, 2018

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

PCR AMPLIFICATION BY TWO DIFFERENT PRIMERS FOR EARLY DIAGNOSIS OF SCHISTOSOMA MANSONI INFECTED SNAILS

***Magda, A. Elsettawy and Marwa A. Salama**

Department of Medical Parasitology, Faculty of Medicine, Zagazig University, Egypt

ARTICLE INFO

Article History:
Received 09th March, 2018
Received in revised form
16th April, 2018
Accepted 29th May, 2018
Published online 30th June, 2018

Key words:
Biomphalaria alexandrina, PCR, 121bp primer, 247bp primer, Sharkiya. Dakhlia, Egypt

ABSTRACT

The traditional methods for detection of snail infections cannot differentiate between animals and human *Schistosoma* and cannot detect early infection so the need for PCR is obligatory. In the present study, two types of primers for conventional PCR were used and compare between them and other traditional methods (shedding, crushing) in attempt to apply this technique in the field for early diagnosis of *Schistosoma* infection as a trial for better control and prevention of the disease. To fulfil this aim in our study we use two main groups of snails the laboratory groups (L) and the environmental groups (E) collected from water canal in Dakhalia and Sharkiya governorate. All laboratory groups are examined by PCR by using two different primers (121bp, 247bp). All environmental snails are examined for infection by shedding, crushing methods then PCR amplification by two different primers (121bp, 247bp). for detection of infection and the obtained results are compared. DNA was extracted from laboratory infected snails in addition to non infected (as negative control) and subjected to PCR using121 bp primers and 247 bp primers which specific to a partial sequence of *S. Mansoni* aldolase. The percentage of detection of infection in the laboratory snail were 53,3%, 66,6%, and 73.3%by using of 121bp primer at the 1st, 5rd, and 8thday of infection respectively, however the detection were 86.6%,73.3% and100% by using of 247bp primers. Regarding the environmental snails,on comparing the sensitivity and specificity of crushing method in relation to PCR amplification using 247bp primer in Sharkiya, were found to be 36.3%, 100% respectively while in Dakhalia were found to be 28.5%,100% .the average sensitivity of PCR amplification by 121bp compared to PCR amplification by 247bp was 63.3%. In conclusion, PCR is superior to the conventional methods and can detect positive cases that were negative when examined by shedding or crushing methods. 247bp primer are more specific than 121bp primer for detection of infection in *S. Mansoni* infected snail and can detect early snail infection and this help control of the disease.

Citation: Magda, A. Elsettawy and Marwa A. Salama, 2018. "Pcr amplification by two different primers for early diagnosis of schistosoma mansoni infected snails", *International Journal of Current Research*, 10, (06), 70818-70823.

INTRODUCTION

In Egypt *Biomphalaria alexandrina* has historically been implicated in the transmission of *S. mansoni*. This snail species is found in the Nile River basin (DeJong *et al*., 2001).The presence of susceptible *Biomphalaria*, is obligatory for transmission of *S. mansoni* in human populations (Negrao-Correa *et al.* 2012). *B. alexandrina* were reported to be widespread throughout the Nile Delta *(*Yousif *et al*., 1998a and Kristensen *et al*., 1999).The development of *S. Mansoni* inside the intermediate host starts immediately after the active penetration of the snail by the miracidium. After penetration, the parasite transformed into primary sporocyst that generate secondary ones and 4 weeks postinfection, cercariae are released from daughter sporocysts and are released from the snail (Negrao-Correa *et al*., 2012).Control of schistosomiasis is mainly depend on repeated population based chemotherapy to reduce intensity of infection but this method requires regular , sustained treatment (Steinauer *et al*., 2008).Snail control is one of the most rapid and accurate means for reducing spread of *S. mansoni* infection (Webbe 1965). Despite efforts to control this disease, based on treatment of infected people and elimination of snails, the level of incidence has shown no significant decrease and continues to spread to new geographic areas particularly in sub- Saharan Africa (Mahfouz *et al*., 2011*)*. The conventional techniques (shedding and crushing) for identification of infected snails are simple and cheap but time consuming, need trained personnel and an appropriate laboratory structure (Schmitt *et al*., 2002 and WHO, 2004).

****Corresponding author:*** **Magda, A. Elsettawy,**
Department of Medical Parasitology, Faculty of Medicine, Zagazig University, Egypt.
DOI: https://doi.org/10.24941/ijcr.30517.06.2018

Therefore, they are highly inaccurate in detection of snail prepatent infection (Caldeira *et al*., 2004).Sandwich ELISA was also used to detect schistosomal antigens in snails' hemolymph two weeks post infection; this time coincides with the formation of daughter sporocysts in the infected snails, the detected antigens increases as infection progresses from prepatency to patency and reached the highest values at 5 and 6 weeks post infection (Abu El Einin *et al*., 2009).Molecular diagnosis of prepatent schistosome infection in snails by the polymerase chain reaction (PCR) has been used for studying human-to-snail transmission, infection prevalence and average intensity in human (Abbasi *et al*., 2010).Several methods were developed depending on detection of specific sequences in genomic DNA.

The use of PCR amplification of the minisatellite repeat from *S. Mansoni* mitochondrial DNA was proposed to identify infected *B. glabrata* snails from one week after exposure to miracidia, and distinguished *S. mansoni* among other trematode (Jannotti-Passos *et al*., 1997). Some PCR methods which depend on detection of repetitive sequences using PCR were sensitive enough to detect early infection in *Biomphalaria glabrata* (Hamburger *et al*. (1998a). Abu El Einin *et al*. (2009) use nested PCR method for detection of infected snails using primers specific to *S. mansoni* fructose 1,6- biphosphatealdolase (SMALDO,247bp) gene, these primers are specific only for *S. mansoni* or molluscan aldolases .In the present study, two primers were evaluated for detection of *S. mansoni* infected *B.alexandrina* snails by PCR. the first one, 121 bp primer , the second one, 247bp primer .

MATERIALS AND METHODS

Type of study: Experimemental case control study, performed at Faculty of medicine Zagazig university in Parasitolology department and molecular biology unit from January2017 to february2018.

Experimental design: In this study, two groups of *Biomphalariaalexandrina* snails were examined, the laboratory groups (L) and the environmental groups (E). Sixtylaboratory non infected snailsthat were purchased from Theodor Bilharz Research Institute, Cairo, Egypt and subdivided into four groups (15, each group). The snails were exposed to infection in Parasitology lab, Snails were infected with eight miracidia/snail with the aid of dissecting microscope, 8 active miracidia were placed into each vial of a special glass plates with 24 well vials then filled with dechlorinated tap water kept under illumination at room temperature for overnight (Prah & James, 1977). The first group , non infected and act as healthy control (L0) the second group infected and examined after one day(L1), the third group infected and examined after 5 day(L5) and the fourth group infected and examined after eight day (L8).The environmental groups includes two groups(150 each group) group I collected from Sharkiyiagovernoratecanals, group II snails collected in the spring from Dakhlia canals, Egypt using the dip net method and during the collection and transportation, snails were kept in a glass container filled with a few amount of water from the same water canal(Yousif *et al*., 1992). All environmental snails are identified morphologically then examined for infection by shedding according to Liang *et al*.(1987) and crushing methods according to Haroun, (1996), then all snails (including the laboratory and the environmental groups) are examined by PCR (two types of primers)

DNA extraction and PCR: The DNA was extracted by lysis of snail tissue in lysis buffer. DNA extraction kit provided from DNeasy® Blood & Tissue Kit, QIAGEN®, Germany.

1- PCR (primers: 121-basepair): The primers designed based on the 121-basepair (bp) highly repeated sequence of *S. mansoni,*Primers were synthesized with DNA synthesizer (OLIGOS, Fenland). *Sm* R 5'GGTGA CCTGCCTA AA AATAC-3'. *Sm* f FTCGTTGT TATCTCCG-3' (Hamburger *et al*., 1998a).

2- PCR(primer: 247-basepair): A partial sequence (274bp) of *Schistosomamanoni* DNA was amplified by PCR using the following primers; f. primer: 5′ TCGTCGTCTGTACCGCCAGC 3′and R. primer: 5′ AGCGAAGC GGCATCCAAGTCT(GenBanklotnumber L38 658) (El-Dabaa*et al*., 1998). Intron's Maxime PCR PreMix Kit is a ready-to-use mixture ofi-Taq ^{TM} DNA Polymerase (5U/µl) :2.5 U.dNTPs : 2.5mM each. Reaction Buffer (10 X): 1x.Gel loading buffer.

Procedure of PCR (according to the manufacturer′s guide in the kit): The PCR amplification reaction was performed in a final volume of 20µl in 0.2 ml PCR tubes. The amplification was carried out in Biometra TG radient Thermal cycler according to (Intron Biotechnology. Inc.) Template DNA(2ul) and primers (2µl) were added into Maxime PCR PreMix (10 µl) tubes. Distilled water was added into the tubes to a total volume of 20µl. The reaction was carried in 35 cycles as follow, Initial denaturation at 94°C for 3 minutes. Annealing at58°C for 20 seconds. Extension at 72°C for 30 seconds. Amplified PCR products (121bp) with 121 bp primer and Amplified PCR products (247bp) by247bp primerwere analyzed by electrophoresis on 1.5 % agarose agar, stained by ethidium bromide (0.5 µg /ml in the running buffer) then, visualized on an ultraviolet transilluminator.

Statistical analysis: Data were analyzed using SPSS .Result were considered significant if P value less than 0.05.The sensitivity is the probability that the test will be positive when the infection is present. The specificity is the probability that the test will be negative when the infection is absent.

RESULTS

As regard detection of infection in laboratory snail by PCR amplification by two different primers 121bp primer and 247bp primer. Out of 15 snail testedone day post infection 8 snails were found to be positive by121bp PCR(53.3%) while 13positive by 247bp PCR(86.6%).Five day post infection out of 15 infected snail the detection of infection were 66.6 %,73.7%by amplification by 121bp primer and 247bp primer respectively. Eight day post infection out of 15 infected snail the detection of infection were 73.3 %,100%by amplification by 121bp primer and 247bp primer respectively (Table 1). Regarding detection of infection in environmental snails, out of 150 snails tested 2,1 snails were found to be positive by shedding method in Sharkiya and Dakhalia governorates respectively with no significant difference. Out of 150 snails tested 4,2 snails were found to be positive by crushing method in Sharkiya and Dakhalia governorates respectively with no significant difference .However out of 150 snails tested 7,4 snails were found to be positive by PCR amplification using 121bp primer in Sharkiya and Dakhalia governorates respectively with significant difference (<0.01).

Table 1. Comparison between PCR amplification by 121bp primer and 247bp primer in detection of infection in the laboratory snails.

Control groups	No.	PCR(121bp)			PCR(247bp)		
		-ve No.	+ve No.	+ve %	-ve No.	+ve No.	+ve%
L0	15	15	0	0%	15	0	0%
L1	15	7	8	53.3%	5	13	86.6%
L5	15	5	10	66.6%	4	11	73.3%
L8	15	4	11	73.3%	0	15	100%

Table 2. Comparison between shedding, crushing and PCR amplification by (121bp primer and247bp primer) in detection of infection in environmental snail in Sharkiya and Dakhalia governorate

overonorate	No.	Shedding		Crushing		PCR(121bp)		PCR(247bp)	
		No-ve	No.+ve.	No-ve.	No+ve	No-ve	No.+ve.	No-ve	No.+ve..
Sharkiya	150	148	2(1.3%)	146	4(2.6%)	143	7(4.6%)	139	11(7.3%)
Dakhlyia	150	149	1(0.6%)	148	2(1.3%)	146	4(2.6%)	141	9(6%)
P value		>0.05*		>0.05*		<0.01*		<0.01*	

Table 3. Sensitivity and specificity of crushing method compared to PCR 247bp in detection of infection in environmental snail

		247bpPCR		Total	Sensitivity	specificity
		Positive	Negative			
Crushing Sharkiya	Positive	4	0	4	36.3%	100%
	Negative	7	139	146		
	Total	11	139	150		
Crushing Dakhliya	Positive	2	0	2	28.5%	100%
	Negative	5	143	148		
	Total	7	143	150		

Table 4. Sensitivity and specificity of 121pb PCR amplification compared to 247bp PCR amplification in Sharkiya governorate the Sensitivity = 63.3%, Specificity= 100%

		PCR 247bp		Total
		Positive	Negative	
121pb PCR Spring season	Positive	7	0	7
	Negative	4	139	143
	Total	11	139	150

Alsoout of 150 snails tested 11,9 snails were found to be positive by PCR amplification using247bp primer in Sharkiya and Dakhalia governorates respectively with significant difference (<0.01) (Table 2). On comparing the sensitivity and specificity of crushing method in relation to PCR amplification using 247bp primer in Sharkiya,were found to be 36.3%, 100% respectively while inDakhalia were found to be 28.5%,100% (Table 3).On comparing the sensitivity and specificity of PCR amplification using 121bp primer in relation to PCR amplification using 247bp primer in Sharkiya,were found to be63.3%, 100% respectively (Table 4).

DISCUSSION

Schistosomiasis is one of the most significant public health problems in Egypt and many efforts had been done for control the disease (Fenwick *et al*., 2006).In Egypt, Biomphalariaalexandrina serves as an intermediate host for S. mansoni and invades many irrigation areas leading to infection of healthy population (Hamed, 2010). The most widely used methods for assessing schistosomiasisendemicity are relying on detection of eggs in stool or urine (Rabarijaona *et al*., 2003). Various monitoring approaches have been tried to help human testing such as examination of human water supply for contamination by human excreta and detection of snail infection by shedding (Vercruysse *et al*., 2001 and Aoki *et al*., 2003).

However, cercarial shedding can be highly focal , low frequent and cannot detect early snail infection (Kariuki *et al*., 2004).The conventional methods as shedding and crushingused for the detection of Schistosoma infected snails are time consuming, and death of the molluscs after collection may occure resulting in underestimation of infection (Abath *et al*., 2006). Molecular techniques for diagnosis of S. Mansoni infection in snail has been developed to overcome the disadvantage of the conventional techniques (Caldeira *et al*., 2004).Moreover PCR enables identification of a large proportion of infected snails especially when cercarial shedding is rare (Abbasi *et al*., 2010). In this study the utility of conventional PCR as a method for the early detection of *S.mansoni* infection in snails in the prepatent period in attempt to apply this technique in the field for early diagnosis of *Schistosoma* infection as a trial for better control and prevention of the disease. The present study was carried out on 210 snails, in two main groups, laboratory and field snails. The laboratory group includes 60 snails; all are about 4-6 weeks of age, which then were subdivided into four sub groups: 15 non infected control snails (L0) and 15 infected snails examined one day after infection (L1), 15 examined five days after infection (L5) and 15 examined 8days after infection (L8). All infected snails were individually exposed to infection by freshly hatched miracidia. All groups were examined by two conventional primers PCR,121bp and 247 bp. The field group consists of 150 snails collected in spring from Sharqia, Egypt, and examined for infection by shedding method, crushing

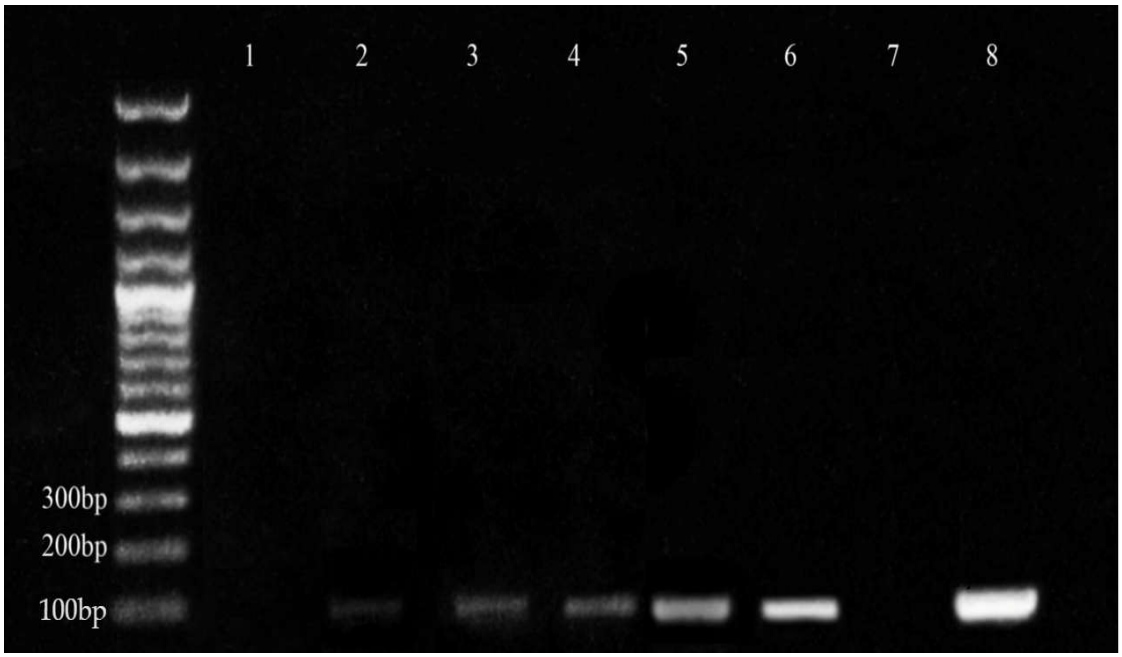

Fig. 1. Showing Agarose gel electrophoresis for PCR amplification of 121 bp of *S.mansoni*:Lane 0 represents 100 bp ladder. (Lane 2) is for (control +ve), (Lane 1) represents control negative snails. Lanes 3, 4, 5 and 6 represent PCR positive product .Lane 7 negative product

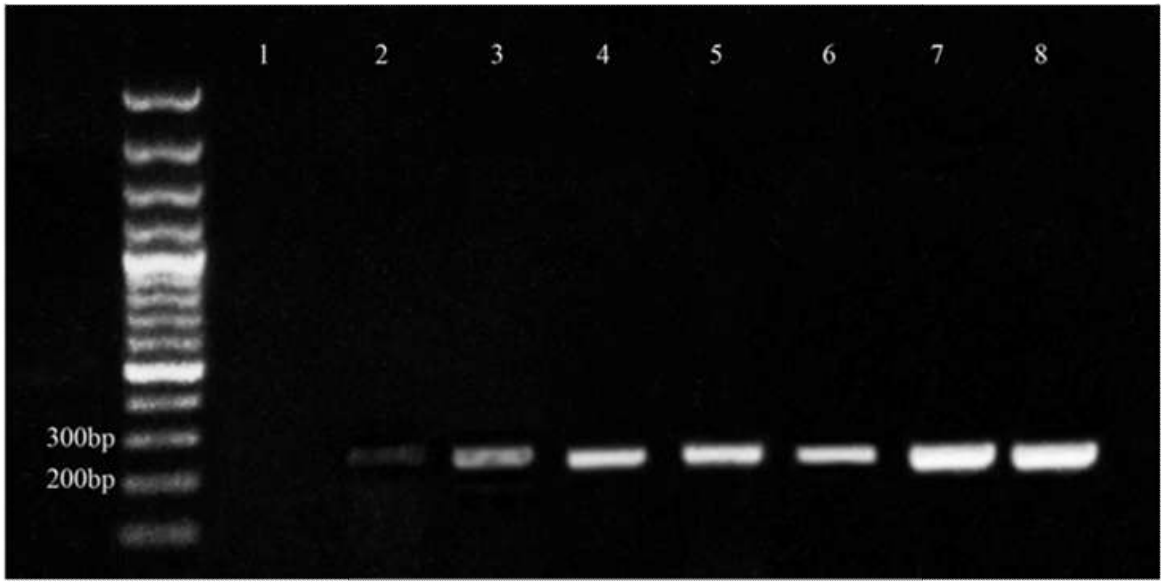

Fig. 2. Showing Agarose gel electrophoresis for PCR amplification of 247 bpof*S.mansoni*: Lane 0 represents 100 bp ladder. (Lane 2) is for (control +ve), (Lane 1) represents control negative snails. Lanes 3, 4, 5,6,7 and 8 represent PCR positive product.

method, and then all snails were amplified by121bp PCR and 247 bpPCR. The collection of the snails in these seasons was in agreement with *Yousif et al. (1999)* who stated that infected *B. alexandrina*s nails fluctuated seasonally in Egypt with minor peak in August and a higher peak in November. Our findings obtained from the laboratory snails as shown in tables (1) showing that etection of the infected snail could be possible early in infection (after one day from exposure to miracidia), as regard using PCR amplification by121bp primer, we found that 8out of 15 snails tested one day after infection (53.5), however 13snail out of 15 (86.6%)by 247bp PCR. five days after infection 10 out of 15 (66.6%)by121bpPCR however 11snail out of 15 (73.3%)by 247bp PCR, eight days post infection, 11 out of 15 (66.6%) however 15snail out of 15 by 247bp PCR gene, and complete negativity of the control snails (L0) (100%).

This result in agreement with *Hamburger et al. (1998a)* who amplified a 121 bp, this gene characterized by repetitive region from *schistosoma* and detected its presence in infected *B. glabrata*snails. The difference between esults may be due to gene sequence. Abu El Einin *et al*. (2009) use a more specific 247 bp primer while Hamburger *et al*. (1998a) depends on121bp, a repetitive gene that constitutes about 10% of *Schistosoma mansoni* genome. In the current study the best result obtained by PCR amplification by 247 bp primers at eighth day post infection(100% positivity) ,this in agreement with Farghaly *et al,*(2016)who used the same primer and detect infection at the 1st,3rd an 7th day of infection70, 85.100% respectively. On the other hand Hanaa *et al,* (2009) were able to detect infection by using the same primer with 100% positivity at the 3rd day post infection. This difference may attributed to using nested PCR instead of conventional PCR.

As regards the environmental snails, all snails were examined by the conventional methods of examination for infection (shedding and crushing) and then subjected to PCR (121 bp and247 bp).The percentage of detection of snail infection by shedding less than crushing, but the best result obtained by PCR amplification by 247bp(100%), this in agreement with Caldeira *et al*,(2004) who stated that shedding and crushing cannot detect infection in dead snails nor in the pre-patent period. In the latter, infection diagnosis is only possible after the parasite has completed its life cycle, when cercariae release is started. No significant difference between Sharkiya and Dakhlia in detection of snail infection by crushing and shedding, while detection of infection by PCR amplification using 121bp and 247bp, there are significant difference (<0.01) between Sharkiya and Dakhlia. Our finding as shown in tables (2) showed that PCR amplification by 247bp primer was superior to the PCR amplification by 121bp primer and can detect positive cases that were negative when examined by shedding or crushing method. This was in agreement with *Melo et al. (2006)* who found that PCR allowed for the detection of infection in pools that were negative by shedding and crushing, They explained this by the fact that PCR can detect prepatent snail infections. Moreover, Farghaly *et al*, (2016) reported that PCR amplification using 247 bp primer is superior to other methods and can detect infection in areas of high transmission and help planning of prevention and control of snail infection by *S. mansoni*

Conclusion

The results obtained in the present study revealed that, all samples positive by conventional methods were also positive by PCR technique, but PCR revealed also other positive cases among the groups that were previously negative when examined by the conventional methods. New molecular amplification by 247bp primer offers more detection of infection in environmental snail .Use of these sensitive and specific techniques will provide essential data for precise determination of the exact time and prevalence of snail infection.

REFERENCES

Aal , Sara A. Abdel-Rahman and Marwa A Salama, 2016. Molecular approach for detectingearly prepatent *Schistosomamansoni* in fection in snail, *J. Parasit. Dis.* 40(3):805-812.

Abath, F. G., Gomes, A. L., Melo, F. L., Barbosa, C. S. and Werkhauser, R. P. 2006. Molecular approaches for the detection of *Schistosomamansoni*: possible applications in the detection of snail infection, monitoring of transmission sites and diagnosis of human infection. Mem. Inst. Oswaldo. Cruz. Rio de Janeiro, 101(Suppl. I): 145-148.

Abbasi, I., King, C. H., Muchiri, E. M. and Hamburger, J. 2010. Detection of *Schistosomamansoni* and *Schistosomahaematobium* DNA by Loop-Mediated Isothermal Amplification: Identification of infected snails from early prepatency. *Am. J. Trop. Med. Hyg.,* 83(2): 427–432.

Abu El Einin, H. M., Mansour, W. A. and El-Dabaa, E. 2009. Assessment of infected *Biomphalariaalexandrina* snails by detecting *Schistosomamansoni* antigen and specific gene. *Aus. J. Basic & App. Sci.3*(3)2747-2753.

Adel Farghaly, Ayman A. Saleh, SoadMahdy, Dalia Abdel KhalikNaglaa F. Abd El-

Aoki, Y., Sato, K., Muhoho, N. D., Noda, S. and Kimura, E. 2003. Cercariometry for detection of transmission sites for schistosomiasis. Parasitol. Int., 52:403 – 408.

Caldeira, R. L., Jannotti-Passos, L. K., Lira, P. M. and Carvalho,O. S. 2004. Diagnostic of *Biomphalaria*Snails and *Schistosomamansoni*: DNA Obtained from Traces of Shell Organic Materials. Mem. Inst. Oswaldo. Cruz.,Rio de Janeiro, 99(5): 499-502.

Caldeira, R. L., Jannotti-Passos, L. K., Lira, P. M. and Carvalho, O. S. 2004. Diagnostic of *Biomphalaria*Snails and *Schistosomamansoni*: DNA Obtained from Traces of Shell Organic Materials. Mem. Inst. Oswaldo. Cruz.,Rio de Janeiro, 99(5): 499-502.

De Jong, R. J., Morgan, J. A., Paraense, W. L., Pointier, J. P., Amarista, M., Ayeh-Kumi, P. F., Babiker, A., Barbosa, C. S. *et al*. 2001. "Evolutionary relationships and biogeography of *Biomphalaria* (*Gastropoda*: *Planorbidae*) with implications regarding its role as host of the human bloodfluke, *Schistosomamansoni*". Mol. biol. & evol., 18 (12): 2225–2239.

El-Dabaa, E., H. Mei, A. El-Sayed, A.M. Karim, H.M. Eld e s oky, F.A. Fahim, P.T. LoVerde and M.A. Saber, 1998. Cloning and charact e rization of *Schistosomamansoni*fructose-1,6-bisphosphatealdolaseisoenzyme. *J. Paras itol.,* 84: 954 - 960.

Fenwick, A., Rollinson, D. and Southgate, V. 2006. Implementation of human schistosomiasis control: challenges and prospects. *Adv.Parasitol.* 61:567-622.

Hamburger, J., Xin, H. X., Ramzy, R. M., Jourdane, J. and Ruppel, A. 1998a. A polymerase chain reaction assay for detecting snails infected with Bilharzia parasites (*Schistosomamansoni*) from very early prepatency. Am. *J. Trop. Med. Hyg.,* 59(6): 872-876.

Hamburger, J., Yu-Xin, X., Ramzy, R. M., Jourdane, J. and Ruppel, A. 1998b. Development and laboratory evaluation of a polymerase chain reaction assay for monitoring *Schistosomamansoni* infestation of water. *Am. J. Trop. Med. Hyg*., 59:468–473.

Hamed, M. A. 2010. Strategic control of schistosome intermediate host. *Asian J. Epidemiol.,* 3(3): 123-140.

Hanaa M. Abu El Einin, Wafaa A. Mansour and Ehab El-Dabaa 2009. Assessment of Infected *Biomphalariaalexandrina* Snails by Detecting *Schistosomamansoni* Antigen and Specific Gene. *Australian Journal of Bas ic and Applied Sciences*, 3(3): 2747-2753.

Haroun, N. H. 1996. differences in susceptibility of *Biomphalariaalexandrina* to *Schistosomamansoni* from Giza and Dakahliagovernerates, Egypt. *J. Egypt. Soc. Parasitol.,* 26(2):327- 335.

Jannotti-Passos , L .K., Vidigal, T.H., Dias -Neto, E., Pena, S.D., Simpson, A.J., Dutra, W.O., Souza, C.P., Carvalho-Parra, J. F. 1997. PCR amplification of the mitocondrial DNA minisatellite region to detect *Schistosomamansoni* infection in *Biomphalariaglabrata* snails. Parasitol., 83: 395-399.

Kariuki, H. C., Clenon, J. A., Brady, M. S., Kitron, U., Sturrock, R. F., Ouma, J. H., Ndzovu, S. T., Mungai, P., Hoffman, O., Hamburger, J., Pellegrini, C., Muchiri, E. M. and King, C. H. 2004. Distribution patterns and cercarial shedding of *Bulinusnasutus* and other snails in the Msambweni area, Coast Province, Kenya. *Am. J. Trop. Med. Hyg.,* 70:449 – 546.

Kristensen, T. K., Yousif, F. and Raahauge, P. 1999. Molecular characterization of *Biomphalaria*spp in Egypt. *J. Mollus. Stud.,* 65*:* 133–136.

Liang,Y. S., John, I., Bruce, J. I. and David, A. B. 1987. Laboratory cultivation of schistosome vector snails and maintenance of schistosome life cycle. Proc. First Sine. *Am. Symp.,* 1:34.

Mahfouz, A., Mahana, N., Rabee, I. and El Amir, A. 2011. Evaluation of Different Immunological Techniques for Diagnosis of Schistosomiasis *haematobium* in Egypt. Life Science Journal, 8(4):858867.

Melo, F. L., Gomes, A. V., Barbosa, C. S., Werkhauser, R. P. and Abath, F. G. C. 2006 Development of molecular approaches for the identification of transmission sites of schistosomiasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 100: 1049- 1055.

Negrao-Correa, D., Mattos, A. C., Pereira, C. A., Martins-Souza, R. L. and Coelho, P. M. 2012. Interaction of *Schistosomamansoni* sporocysts and hemocytes of *Biomphalaria.* J. Parasitol. Res., 2012:743-920.

Prah, S. K. and James, C. 1977. The influence of physical factors on the behavior and infectivity of miracidia of *Schistosomamansoni* and *Schistosomahaematobium.* II-effect of temperature and ultraviolet light. Helminthol., 51: 73-85.

Rabarijaona, L. P., Boisier, P., Ravaoalimalala, V. E., Jeanne, I., Roux, J. F., Jutand, M. A. and Salamon, R. 2003. Lot quality assurance sampling for screening communities hyperendemic for *Schistosomamansoni*. *Trop. Med. Int. Health.,* 8:322 – 328.

Schmitt, J., Wuhrer, M., Hamburger, J., Jourdane, J., Ramzy, R. M. and Gever, R. 2002. *Schistosomamansoni* and *Schistosomahaematobium:* identification and characterization of glycoconjugate antigens in hemolymphof infected vector snails. *J. Parasitol.*, 88: 505513.

Steinauer, M. L., Agola, L. E., Mwangi, I. N., Mkoji, G. M. and Loker, E. S. 2008. Molecular Epidemiology of *Schistosomamansoni*. *Infect. Genet. Evol.,* 8(1): 68–73.

Vercruysse, J., Shaw, D. J. and Bont, J. 2001. Index potential contamination for schistosomiasis. Trends Parasitol., 17*:* 256 – 261 .

Webbe, G. and James, G. 1971. The importance and maintenance of schistosomes of human and veterinary importance. 9th symposium of British Society of Parasitology, P: 77.

World Health Organization, 2004. World Health Organization Special Programme for Research and Training in Tropical Disease. TDR home page at http://www.who.int/tdr/

Yousif, F. H., Magdy, T. K., El- Emam, M. A. 1992. Evaluation of three common tools in estimating *Biomphalriaalexandrina* snail population in irrigation ditches. *J. Egypt. Soc. Parasitol.,* 23(1): 195-211.

Yousif, F., El-Emam, M., Abdel-Kader, A., El-Din, A. S., ElHommossany, K. and Shiff, C. 1999. Schistosomiasis in newly reclaimed areas in Egypt. 2 – Patterns of transmission. *J. Egypt. Soc. Parasitol,* 29:635-648.

Yousif, F., Ibrahim, A. and El-Bardicy, S. N. 1998 Compatibility of *Biomphalariaalexandrina, Biomphalariaglabrata* and a hybrid of both to seven strains of *Schistosoma mansoni* from Egypt. J. Egypt. Soc. Parasitol.28:863-881.

****