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Prevalence of urinary schistosomiasis and Infections withTrematode larval stages in *Bulinus truncatus* Snails from Qena Upper Egypt

[1,2]Abdel-Nasser A. Hussein and [2]Saad M. Bin-Dajem

[1]Zoology Department, Faculty of Science, South Valley University. Qena, Egypt.
[2]Biology Department, Faculty of Science, King Khalid University. Abha, Saudi Arabia.

Abstract: To detect the prevalence of urinary schistosomiasis and *Bulinus truncatus* snail, 150 urine samples and 837 *B. truncatus* snails were collected from Qena Governorate in Upper Egypt. Infection rates were 15.7% (age-period 8-17 years) among males and 20.7% among females (age-period 9-14 years). The highest collected snails number was found in January and seasonally in autumn, but November and winter have the highest infection- peak. Generally, 1.9% of *B. truncatus* had been harboured one or more trematode larvae. Schistosome infection was found in 8 (0.96%) and 6 (0.72%) as single and double infection. Echinostome cercariae and xiphidiocercariae have represented as other trematodes infection.

Key words:

INTRODUCTION

Schistosomiasis: is the most important helminth-parasitic disease of human and domestic livestock. Schistosomiasis is in focus of WHO because of its socioeconomic and public health importance in tropical and subtropical areas, morbidity and mortality and cercariae are agents of swimmer's itch. Also, it has an especially detrimental effect on the growth and development of school-age children. It is easily transmit into developed countries from developing countries, in which it is an endemic disease. *Bulinus* species snails are well-known intermediate hosts of urinary tract. **schistosomiasis:** in Egypt. The epidemiology of schistosomiasis in Egypt over a 5000 year period. Evidence from mummies demonstrates the presence of this disease in ancient Egypt[4,7,23]. **Schistosomiasis** has been shown to be a major social and medical problem in women (Genital schistosomiasis**)**, possibly enhancing the transmission of HIV. Other organs (such as lungs, spinal cord, and brain can also be involved. The similarities of symptoms are problematic because of the sensitivity in sexually transmitted diseases[12,2]. Also those eight cases of spinal cord schistosomiasis reported in Egypt. Diagnosis was established by identification of ova in histopathologic studies[28]. Up to 75% of women with urinary tract schistosomiasis have *S. haematobium* ova in the genitals[22].

Human infection, an estimated 200 million people actually infected with bilharzias in 74 countries in Africa, the Middle East, South America and South East Asia, and in addition to more than 600 million people are at risk[6,31,27]. About six million Egyptians of the rural population have the infection[8]. Among humans *S. mansoni* infections are 21.5% and 13.8% of males and females, respectively[17]. Thus it remains one of the most serious public health problems in rural Egypt. The excretion of eggs of *S. haematobium* in 1,400 boys living in three villages in Upper Egypt had been studied[21]. Two large villages were chosen in Menoufia Governorate (El-Garda village & Salamoniya). As a whole, schistosomiasis (*S. mansoni*) infection rates were much higher in Salamoniya village (27.9%) and 8.1% in El-Garda[20].

A survey for *S. haematobium* in the village of El-Gezira El-Shakra and pupils enrolled in the El-Gezira El-Shakra village primary school, El-Saf district in the Upper Egypt Giza Governorate. Urine was screened by a polycarbonate filtration method. The prevalence of *S. haematobium* infection in the population sample and the school children was 7.4% and 10.6%, respectively. The prevalence of infection was highest in the younger age groups, and males (10.6%) were infected more than females (4.3%)[29]. Health questionnaires and parasitologic examinations of urine and stool samples were evaluated from a stratified random sample of 89, 180 individuals in 9 governorates of Egypt to investigate the prevalence of infection with *Schistosoma* sp. in Egypt. Prevalence of *S. haematobium* in 4 governorates in Upper Egypt in which it is endemic ranged from 4.8% to 13.7% and

Corresponding Author: Abdel-Nasser A. Hussein, Biology Department, Faculty of Science, King Khalid University. 9004 Abha, Saudi Arabia.
Mobile: +966/555835233, Fax +966/7/2290165, E-mail: Abdelnasser@lycos.com

averaged 7.8%. Age stratified prevalence of infection peaked at 15.7% in the 10-14-year-old age-group and decreased to 3.5-5.5% in all groups more than 25 years of age. Males had higher infection rates than females in all age groups. The prevalence and intensity of infection with *S. haematobium* was low in endemic Upper Egypt governorates[9]. One of the most important study has strongly mentioned the situation of schistosomiasis (*S. mansoni*), in Nile Delta of Egypt, but no work was done about *S. haematobium* during the mentioned study[10].

Qena Governorate is one of the included areas in a big research-project study of epidemiology of schistosomiasis, 34 ezba (small hamlet) and ten villages. Parasitologic examination of urine and stool were made for *S. haematobium* and *S. mansoni*, respectively. The overall estimated prevalence of *S. haematobium* was 4.8 ± 0.7%. Considerable variation in prevalence was observed between the villages and ezbas, ranging from 0.0% to 20%, with the smaller ezbas having a slightly higher overall prevalence. The age- and sex-specific patterns of *S. haematobium* showed -in early adolescence- males have a higher prevalence than females[15]. A study of *S. mansoni* infections among humans and prevalence between *Biomphalaria alexandrina* snails revealed 0.48% infection among snails and **21.5% and 13.8**% among males and females, respectively[17].

Fresh-water snails are the intermediate hosts of schistosomiasis, studies deal with the infection are very rear; *B. truncatus* (Audouin) were infected with schistosome parasites. Infected *B. truncatus* occurred in 2 (4.8%) of the 42 sites surveyed. Snail infection was highest (6.2%) from May to July and lowest (3.0%) from February to April. The number of infected snails increased with density. The snail population peaked in June while the smallest number was collected in March. The seasonality of the snail population was attributed to changes in the rainfall pattern[1]. In the western Cameroon crater lakes, a study to assess the role of snails belonging to the Bulinus sp. that identified as *B. natalensis* or *B. tropicus* in the transmission of urinary tract schistosomiasis in Cameroon. The percentage infection of snails challenged ranged from 03.33 to 06.00% for Nchout Monoun population and from 01.85 to 04.76% for Monoun Ngouondam population. No progeny from Petponoun-East and Petponoun-West were experimentally successfully infected with *S. haematobium*. All the 351 snails dissected were euphallic. Previous malacological surveys revealed the absence of Bulinus sp. naturally infected with human schistosomes. These results suggested that Bulinus sp. was not susceptible to infection with *S. haematobium* in the Cameroon Western highland crater lakes. These observations justify the absence of transmission foci (for urinary tract schistosomiasis) in this area[26]. A total of 2802 molluscs of different species from two fishponds and one swamp in Czech Republic were examined, 951 (33.9%) were found to be infected with larval trematodes of 28 species[11]. In a study carried out on *Biomphalaria alexandrina* snails about infections with *S. mansoni* in Qena, Egypt, 0.48% was the infection percentage out of 2070 snail examined[17].

Very few research papers have been done concerning infections of fresh-water snails in Egypt,[16,18] in Qena;[30] in Giza, Egypt.

To the best of our knowledge, no information are found in the literature on the infection of *B. truncatus* snails especially in Qena Governorate, Upper Egypt, and also study of schistosomiasis that covers nearly all districts of Qena are absent. Inaddition to the social and medical problem of genital schistosomiasis, the present study is aims to accurately predict age/gender specific intensity profiles of endemic urinary tract schistosomiasis infections, and to determine the infection rate and kinds of snail-emerged ceracriae from different localities in Qena Governorate.

MATERIALS AND METHODS

Study Site: The governorate of Qena is located in Upper Egypt, about 608 km at the South of Cairo. It extends over a distance of 240 km (Fig. 1a), and is bordered on the north by the Sohag Governorate, on the south by Asswan, on the east by Red sea and on west El-Wadi El-Gadied. According to estimation 2004, about 2,800 milion people (inhabitants) are living in Qena governorate. Also This Governorate is still nearly virgin of such studies.

Collection and Examination of Urine Samples: Urine samples were collected from seven different localities belongs to four districts as follows: Nag Ahmed Bekheet, Nag El-Rebba, Abu-Tesht city (Abu-Tesht), Gezeret Armant El-Hate (Armant) and El-Shaghab (Isna). In all districts, the main activities are agriculture and pastoralism, but Qena district (El-Tramsa, Qena city) is more urbanized (Fig. 1b). A total of 150 urine samples were collected, including 29 females and 121 males and 10 to 15 year-old subjects in females and 8 to 17 years-old males. A urine sample per subject was collected and personal data (name, sex, and age) were noted on delivery of the container. Urinary tract specimens were transported to the laboratory within 1-3 hours of collection. In the laboratory, each urine sample was concentrated by the sedimentation into sedimentation glass[19].

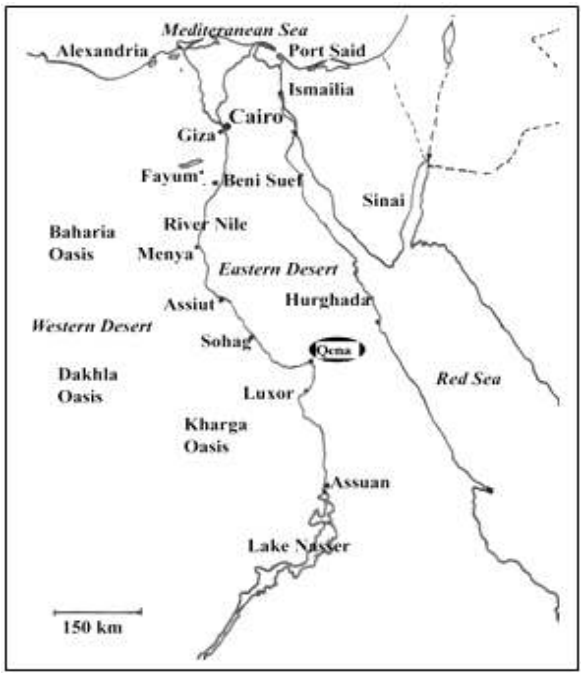

Fig. (a):

Fig. 1(b):

Fig. 1: (a) A map of Egypt showing the position of Qena governorate, and 1 (b) map indicating the four studied districts in Qena (Abu-Tesht, Qena, Armant and Isna) and the location of localities surveyed for human samples.

Numbers indicate the localities in which human stools have been sampled (N = sample size M\F)
1: Nag. Ahmed Bekheet (N=18\8); 2: Nag. El-Rebba (N= 16\3); 3: Abu-Tesht city (N= 19\0)
4: El-Tramsa (N=18\10); 5: Qena city (N=10\3); 6: Gezeret Armant El-Hate (N=17\0)
7: El-Shaghab (N=23\5)

The procedure is repeated until the supernatant fluid is relatively clear. After final removal of the water, a small drop of bottom is removed with a long pipet to a glass slide then examined microscopically, in order to look for schistosome eggs. Since egg production shows a marked seasonality, I did not estimate intensities (i.e. parasite burden per host). Therefore, the only data obtained was the prevalence (i.e. the proportion of infected individuals among the total examined).

Bulinus Snail's Collection: Snails of the genus *Bulinus* were collected from water bodies of 13 different localities:- from north to south: Nag Ahmed Bekhet, Nag El-Rebba, El-Nagma and Houmran (Abu-Tesht district), Nag Hammady city (Nag Hammady district), Dandara, El-Tramsah, El-Sale and Karm-Omran (Qena district), El-Keratia (Qus district), Armant city and Gezerat Armant El-Hate (Armant district), and Nag El-Maala and El-Shaghab (Isna district). Minimum one visit has been carried out for each locality per season. Snail collections were performed following standard malacological methods[24,25]. to assess population densities. A total of 837 *Bulinus* sp snails could be collected.

Collection and Study of Trematode Larval Stages: Cercariae were obtained after natural emergence from collected *B. truncatus* snail individuals placed in natural water in Petri dishes. Non-shedding snails were kept in the laboratory and re-examined weekly to verify that cercariae were not shed by snails which could have been infected only shortly before being collected. When snails proved to be negative after several examinations by the shedding method, they were gently crushed in a Petri dish containing a small amount of dechlorinated water. The fleshy part was removed and was dissected under a microscope. When intramolluscan trematode larval stages were detected in the dissected snails, they were transferred to a glass slide for detailed examination under the binocular microscope. The same process was followed for snails dying or recently died. The number of snails shedding cercariae and those presenting infection by intramolluscan larval stages were recorded. Cercarial types were classified according to[5,14].

Institutional Ethical Review Procedure: The surveys were carried out after informed consent was obtained from the local authorities in the villages, as well as by all participants.

RESULTS AND DISCUSSION

Results:

Human Infection with *S. haematobium:* Suveys were carried out in 7 villages spread along Qena Governorate, Upper Egypt. Those villages are Nag. Ahmed Bekheet, Nag. El-Rebba and Abu-Tesht city (Abu-Tesht district); El-Tramsa and Qena city (qena district); Gezeret Armant El-Hate (armant district) and El-Shaghab (Isna district). Urine specimens were obtained from 150 persons (121 males and 29 females). The estimated prevalence of *S. haematobium* was 19\6 and percentage of 15.7%/20.7% in males/ females (Table 1).

The highest infection rate among districts was found in Qena district, where 25.0%/30.8% had been infected with *S. haematobium*. According to gender, females had been investigated only in Abu-Tesht Qena and Isna districts, where the percentages were 18.2% and 30.8% in the fist two districts, respectively and no infection was encountered in females in Isna district. No female specimens had been examined in Armant district. Among males, 9.4%, 11.3%, 21.7% and 25.0% were found to be infected in Abu-Test, Armant, Isna and Qena districts, respectively.

The highest infection rates among females were found in El-Tramsa (40.0%) and Nag. El-Rebba (33.3%). No infection was encountered in females of El-Shaghab. Males in all screened localities were found harbouring eggs of *S. haematobium,* 33.3%, 21.7%, 11.3%, 11.1%, 10.0%, 10.5% and 6.3% in El-Tramsa, El-Shaghab, Gezeret Armant El-Hate, and Nag. Ahmed Bekheet, Qena city, Abu-Tesht city and Nag.El-Rebba, respectively.

In relation to age-period, according to the following criterion school children (6–11 years old) and adolescents (12–18 years old), the study focused on 8-17 years old that is late school-children and adolescents. Even all ages are susceptible to infection; the age-period 10-15 is the most sensitive period of the Infection with *S. haematobium*, where the highest infection rate (33.3%) among males was recorded in that age-period. In females, the most sensitive age-period is 10-13 years old, where the highest infection rate is 40.0%. The overall intensities of schistosomiasis haematobium by village, according to sex and age groups, are shown in Table 1.

Table 1: The intensities of schistosomiasis haematobium by village, according to sex and age groups (**%** Percentage, ***F*** Female; *M* Male; *No* Number)

District	Locality or Village	No. Examined (M/F)	Infected (M/f)		Age-period
			No	.%	
Abu-Tesht	Nag. Ahmed Bekheet	18/8	2/1	11.1/12.5	10-17/10 -13
	Nag. El-Rebba	16/3	1/1	6.3/33.3	10-16/9-11
	Abu-Tesht city	19/0	2/0	10.5/0.0	8-15
Qena	El-Tramsa	18/10	6/4	33.3/40.0	10-15/10-13
	Qena city	10/3	1/0	10.0/0	10-15/10-15
Armant	Gezeret Armant El-Hate	17/0	2/0	11.3/0.0	10-16
Isna	El-Shaghab	23/5	5/0	21.7/0.0	10-16/10-14
	Total	121/29	19/6	15.7/20.7	

Snail Population Dynamics and General Prevalences: The collected snails are identified as *Bulinus truncatus* (according to Frandsen, 1983 and Brown, 1994). Snail population dynamics studies showed monthly fluctuations in which the number of snails appeared highest in January (n = 215) and September (142), whereas the lowest numbers appeared in July (10) and June (4). Died *B. truncatus* snails (shells) were encountered in December (307), January (170), October (14), November (8) and the lowest number collected was in August (2). The seasonal variation of the snail populations showed that the snail numbers peak in winter (359) and autumn (243), and decrease in summer (126) and winter (109). Most of died snails were collected in winter (477) and autumn (22) (Table 2).

Out of 837 examined snails 16 (1.9%) were found harboured trematode infection. November (7.41%) possesses the highest position in the infection peak, followed by January (3.26%). Seasonally, the highest infection rate was in winter (2.79%) followed by

autumn (1.65%), while in spring and summer only 1 snail was infected in each season with a percentage of 0.92% and 0.79% out of 109 and 126 examined snails, respectively (Table 2).

Single infection is the common among the examined snails, which 10 (62.5%) infected snails out of the total examined number. Double infection was found in 6 (37.5%) infected snails (Table 2).

Infections of Snails with Schistosome Larval Stages: Out of 837 *B. truncatus* snails, 14 individuals (1.67%) were shedding schistosome cercariae. Single infection was encountered in 8 snails (0.96%) and mixed infection with xiphidiocercariae was found in 6 snails (0.72%). Single infections were recorded in Nag El-Rebba (Abu-Tesht district), El-Tramsa (Qena district), El-Shaghab (Isna district), while mixed infection were found in three localities (El- Sale (Qena district), Gezerat Armant El-Hate (Armant district), Nag El-Rebba (Abu-Test district) (Table 2).

A monthly analysis shows that *B. truncatus* snail infection by schistosome cercariae fluctuated between 0.89% and 7.41% in January and November, respectively. An appreciable rise of the infection rates was found in September (1.41%), in February (2.97%), in April (3.03%) and in January (3.26%).

The highest percentage of schistosome infection being recorded during November (7.41%). The single infection by schistosome cercariae was encountered only in three months (January, February, and September) and January possesses the highest infection rate in both single and mixed infections. No schistosome infection could be found in *B. truncatus* collected during December, March, May, June, July and October (Table 2).

A seasonal analysis shows that all *B. truncatus* snails collected during the four seasons were positive for infection with schistosome. The highest infection rate (2.79%) was found in winter (December-February), and the lowest was in summer (June-August) (0.79%). A rise in the infection rate was noticed during summer (June-August) (0.79%) and spring (March-May) (0.92%).

Infections by Other Trematode Larval Stages: During the present study two species of cercariae, other than schistosome, were found among a total of 16 (1.91%) infected snails out of 837 *B. truncatus* individuals examined. Xiphidiocercariae were found in 6 infected snails mixed with schistosome infection (0.72%), no single infection of *B. truncatus* snails by xiphidiocercariae. Only one snail infected by xiphidioceracria was recorded in each of February and April, and two snails in November and January. The infection with xiphidiocerciae was encountered in three localities {El- Sale (Qena district), Gezerat Armant El-Hate (Armant district), and Nag El-Rebba (Abu-Test district)}. Echinostome cercariae were found -singly- in 2 snails (one in February and one in August) (0.24%). In only two localities echinostome cercariae were found (El-Tramsa (Qena district), Nag El-Rebba (Abu-Tesht district)). The sporocysts of both xiphidiocercariae and schistosome cercariae and rediae of echinostome were also found in the respective infected snails.

Table 2: Monthly infection rate of *B. truncatus* with schistosome cercariae (*D Inf* Double infection; *No* Number infected\%; *SInf* Single infection).

Month	Bulinus truncatus				
	Life	Shell	No\%	S Inf \%	D Inf\%
December	43	307	0\0	0\0	0\0
January	215	170	7\3.26	5\71.4	2\28.6
February	101	0	3\2.97	2\66.7	1\33.3
March	51	0	0\0	0\0	0\0
April	33	0	1\3.03	0\0	1\100
May	25	0	0\0	0\0	0\0
June	4	0	0\0	0\0	0\0
July	10	0	0\0	0\0	0\0
August	112	2	1\0.89	1\100	0\0
September	142	0	2\1.41	2\100	0\0
October	74	14	0\0	0\0	0\0
November	27	8	2\7.41	0\0	2\100
Total	837	501	16\1.9	10\62.5	6\37.5

Discussion: It is well known that **urinary tract schistosomiasis** is prevalent along the Nile Valley from Delta region to Upper Egypt. Also *B. truncatus* snails have been recorded in water bodies throughout all Egypt. Papers deals with the infection of the fresh-water intermediate hosts are very rear, especially those focuses on *B. truncatus* in Egypt. A five-year study of the excretion of eggs-mainly- of *S. haematobium* in 1,400 boys living in three village in Upper Egypt) had been done. They stated that no long-term changes infection appears to have occurred and the reduction in parasite density following the intervention appeared to be only temporary[21]. The overall estimated prevalence of *S. haematobium* infection- in the present study-was higher (15.7% in males & 20.7% in females) than recorded in nine Governorates[9], where Prevalence of *S. haematobium* in 4 governorates in Upper Egypt ranged from 4.8% to 13.7% and averaged 7.8%. They considered *S. haematobium* is rare in the studied governorates, so they considered Ismailia had the highest infection rate (1.8%). In A study occurred in El-Gezira El-Shakra El-Saf district in the Upper Egypt Giza Governorate, the prevalence of *S. haematobium* infection in the population sample and the school children was 7.4% and 10.6%, respectively[29]. The estimated prevalence of *S. mansoni* was 30 with overall percentage of 20% in Qena, Egypt[17].

Regarding to Gender: males had higher infection rates than females in all age groups[9]. and the same was mentioned during a study of urinary tract schistosomiasis in Qena[15]. Also the similar result reported in Giza Governorate[29]. Meanwhile the present study revealed a higher infection rate in females than males. These results may be due to cultural and behavioral factors, where females being associated more with the washing of clothes and kitchen utensils in large canals where transmitting infected snails are present. This result agrees with result obtained during study of liver fluke (*Fasciola*) infection[10], where they mentioned that the prevalence of liver fluke infection in females appeared to be significantly higher than in males. The result of big study- of 17.822 individuals carried out in Qena Governorate- was stated overall prevalence of *S. haematobium* was 4.8% and a considerable variation in prevalence was observed between the villages and ezbas, ranging from 0.0% to 20%, with the smaller ezbas having a slightly higher overall prevalence. Simillar result was obtained in Qena, Egypt that males (21.5%) had higher *S. mansoni* infection rates than females (13.8%)[17].

Concerning Age-period: the specific age of urinary tract schistosomiasis is ten years old[29]. The age specific patterns of *S. haematobium* showed typical peak prevalence in early adolescence in Qena[15]. Age stratified prevalence of infection peaked at 15.7% in the 10–14-year-old age group[9]. As many studies have been done in different age-groups, the present study focused on 8-17 years and it is not so far from the last age-group, where the highest infection rate (33.3%) among males was recorded in that age-period (10-15). In females, the most **sensitive age-period** is 10-13 years old, where the highest infection rate is 40.0%. In the same time, the present study agrees that all ages are susceptible to infection.

According to[13,3]. the collected snails are belonging to *Bulinus truncatus*, which appears to be distributed throughout the whole country.In Upper Egypt, previous malacological studies had mentioned five kinds of cercariae in *B. truncatus* snails; xiphidiocercaria (1.71%), holostome cercaria (16.91%), *Cercaria pigmentata* (12.02%), *Echinoparyphium recurvatum* (53.04%) and cercaria of *S. haematobium* (0.40%)[16]. In Africa, Nigeria, 4.8% *B. truncatus* snails infected[1]. reported, a slightly higher percentage (03.33 to 06.00% and 01.85 to 04.76%) had been recorded among *Bulinus* spp. population in two localities in Cameroon[26]. The authors agreed with the previous claim that *Bulinus* sp. was not susceptible to infection with *S. haematobium* in the Cameroon Western highland crater lakes. The present study has recorded a higher percentage (1.9%) infected *B. truncatus* snails with *S. haematobium* than the reported percentage (0.40%)[16]. It is sufficient number to discharge enough cercariae to infected huge number of people.

B. truncatus snail infection was highest (6.2%) from May to July and lowest (3.0%) from February to April in Nigeria. The seasonality of the snail population was attributed to changes in the rainfall pattern[1]. In the present survey, monthly infection fluctuated between 0.89% in January and 7.41% in November. The lowest infection rate was found in September (1.41%), then increased to 2.97% in February and 3.03% in April followed by 3.26% in January. The highest percentage of schistosome infection being recorded during November (7.41%). Seasonally, all *B. truncatus* snails collected during the four seasons were positive for infection with schistosome. The highest infection rate (2.79%) was found in winter and the lowest was in summer (0.79%). Generally those data is slightly higher than those recorded in Jos metropolis (Nigeria)[1]. All the difference in infection rates may be due to the different climatic conditions in Upper Egypt from those in Nigeria

Concerning the Snail Population: in Nigeria, the population of *Bulinus* sp. snail peaked in June while the smallest number was collected in March[1]. January (215) and September (142) possesses the highest collected number in the present study, whereas the lowest number appeared in June (4). Because June in Egypt is the most hot month in summer, it different from June in Nigeria that is rainfall month. This effection of climatic conditions is clear in the seasonal variation of the snail populations in Upper Egypt, where the snails numbers peak in winter (359) and autumn (243), and decrease in summer (126) and winter (109).

B. truncatus is one of the seven Egyptian studied snail species, which were found to be infected by xiphidiocercariae in Giza, Egypt[30]. During the present study two species of cercariae, other than schistosome, were recorded in the population of *B. truncatus*, where xiphidiocercariae were found in six infected snails mixed with schistosome infection (0.72%), but the present study did not recorded any single infection of *B. truncatus* snails by xiphidiocercariae out of 837 examined snails. Echinostome cercariae were found -singly- in 2 snails (0.24%)**.** Echinostome cercariae were found in two localities; El-Tramsa (Qena district) and Nag El-Rebba (Abu-Tesht district).

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