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Original article

Localization of Some Larval Stages of *Schistosoma spp.*, in the Derna Waterfall, Libya

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ABSTRACT

Background and aims*. Schistosoma, one of the Trematoda, is responsible for nearly 80,000 deaths per year. These worms cause the disease Schistosomiasis, which is the most common parasitic disease around the world. Studies on this topic in Libya were found to be very few, practically absent from the site of this study. In addition, lack of consciousness was a valid reason for us to study this topic; this study was aimed at tracing the stages of the life cycle of the Schistosoma worm and recording it in Derna waterfall.* ***Methods****. Random samples of water and mud from waterfall water was subjected for examining directly with a light microscope to search for miracidium phase. Meanwhile, samples of snails were gathered on the waterfall's banks and categorized in an effort to locate the intermediate host. Stool and urine samples were collected from people living around the bank of the waterfall to search for eggs, and they were recorded. Blood samples from people living around the bank of the waterfall were collected and tested using a Schistosoma IgG ELISA to determine if they had antibodies against Schistosoma.* ***Results.*** *Miracidiam stage and eggs was found in water and mud samples in the study area, which indicates the presence of the adult phase. Results from snail samples confirm the presence of intermediate hosts of Schistosoma, including Biomphalaria and Bulins. Meanwhile, the presence of eggs and positive IgG was found among people living in the area of study.* ***Conclusion.*** *Results of this study recommend taking necessary measures include awareness of the population and the elimination of snails that are intermediate hosts of the worm, thus eliminating these worms.*

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INTRODUCTION

Wide Flatworms of the genus *Schistosoma* are parasites (Phylum Platyhelminthes) that currently infect over 200 million people worldwide [1]. Where these worms cause the disease Schistosomiasis, which has been affecting human health for at least 4,000 Years [2]. Characteristic symptoms are described in early Egyptian papyri and analysis reveals immunological cues as to its presence in ancient mummies [3]. The etiology of Schistosomiasis in humans was first discovered in (1799–1801) by Europeans surgeons were with Napoleon's army in Egypt [4], where are record contact with *S.haematobium*. They reported that hematuria (blood yurine) was prevalent among the troops, although the cause of course was unknown. Nothing further was learned about *S. haematobium* for more than 50 years, until a young German parasitology's, Theodor Bilharz, discovered the worm that caused it. He announced his discovery in letters to his former teacher, Von Siebold, naming the parasite Distomum *haematobium.* (Bilharz

died of typhus at age 37.) During the next few years, it was discovered that 30% to 40% of the population in Egypt bore infections of *S. haematobium*, and the worm was even found in an ape dying in London. The peculiar morphology of the worm made it clear that it could not be included in genus Distomum, so in 1858 Weinland proposed the name Schistosoma. Three months later Cobbold named it Bilharzia, after its discoverer. This latter name became widely accepted throughout the world, and the parasite was even given the nickname "Bill Harris" by British soldiers serving in Europe during World War I [4].*S. japonicum* was discovered 1904, though the clinical symptoms had been a described syndrome for more than half a century[5] . *S.mansoni* was described in 1907, and named in the honor of Sir Patrick Manson, the first scientist to speculate that the difference in egg morphology and manner of excretion (terminal versus lateral spine; fecal versus urinary) of African *schistosomes* was due to the existence of two separate species (*S. haematobium and S. mansoni).*

There were some confusion as to how exactly the *Schistosomes* entered their human hosts, and the exact dynamics of *Schistosome* life cycles. but all disagreement was solved in 1913 when Keinosuka Miyairi and Masatsuga Suzuki clearly elucidated transmission and life cycle details for *S. japonicum*[5] .In China in the 1950's, Mao recognized the problem of Schistosomiasis and decided to work towards eradication. He raised public awareness, and funded many public health projects. Mao's efforts in the 1950's helped curb Schistosomiasis infection [6]. *Schistosomes* are trematoda worms ('flukes') belonging to the phylum Platyhelminthes. The adult worms live in the vascular system of birds and mammals ('blood flukes'). All the *Schistosomes* that mature in man belong to the genus Schistosoma of the order Strigeatoidea and the family Schistosomatidae [7].The genus *Schistosoma* contains 19 species [8]. Five of which (*Schistosoma haematobium, S. mansoni, S. japonicum, S. mekongi and S. interatobium*) are of major pathological importance, While the others are essentially parasites of non-human mammals [9]. *Schistosoma Spp* have complex life cycles consisting of both free-living and parasitic forms [10].Also are differ from other Trematodes having separate adult male and female parasites. Sexual reproduction happens in the definitive host (humans, cattle, etc), depending on the *Schistosoma* species and a sexual reproduction phase happens in the snail (intermediate host) cercaria (released by specific snail species the water) [4]. Larvae emerge from the snails and swim in the water until they come into contact with an individual and penetrate the skin. Once inside the body, the larvae develop into male and female warms, which pair up and live together in the blood vessels for years even up to 40 years. Approximately after 6 weeks post infection the adult worm –pairs start to lay egg , which penetrate the intestinal wall (*S. mansoni , S japonicum*) or the bladder wall (*S. haematobium*) which are passed out of the body in the urine and faces. If people urinate or defecate in bodies of freshwater, the eggs migrate to snails where they eventually hatch and begin the cycle again. Some *Schistosoma* eggs, however, remain trapped in the body and migrate to specific organs. (Depending on the type of parasite), where they can inflict major damage [4].This parasitoids is characterized by being initially asymptomatic, but it is able to evolve into more severe clinical forms, potentially causing death [11].Globally, more than 200 million are currently infected in 74 countries [8]. Probably more than 95% of human infections are due to S. *mansoni* and S. *haematobium*. Through my knowledge of the previous studies found in a report of the World Health Organization [12]. This parasite is present in Libya and despite this reference, through the knowledge of previous studies, the last study on this parasite in the city of Derna was in 2012 and until this time, there is no study on this subject, and that gives main reason to start this study to find out Localization some larvae stages of *Schistosoma spp* in the Derna Waterfall.

METHODS

The study area and ethics

The study protocol was reviewed and approved by Libyan National Bioethics Committee for Biosafety and bioethics with Ref No: 46-S2-20. Inclusion criteria involve: patients agreement participation in the study. This study was conducted in the Derna Waterfall, in Al-Jabal Al-Akhdar, south of the city of Derna in the northern Cyrenaica region of Eastern Libya. The falls have a drop of about 20 miters they are located approximately 7 km (3,4 mi)from central Derna ,in the Derna District (22.614614,32.717894) (Figure 1). The Climate in this area varies from hot and highly humid in summer to cold in winter [13]. This area depend on water streams as the main source of water for domestic and irrigation purposes , which create favorable snail breeding condition.

Collection water samples

In the research area, samples were taken from different locations along the Waterfall (nine points of interest.) Between December 2019 and February 2020, five samples of water and mud were taken from each site (Figure 2), samples placed in special packets, numbered according to the collection and send it to the laboratory [14]. Two drops of the specimen put on the slide and examined directly, identified miracidium as defined using an optical microscope and scanning both slides with 10x and 40x through the scan [15].

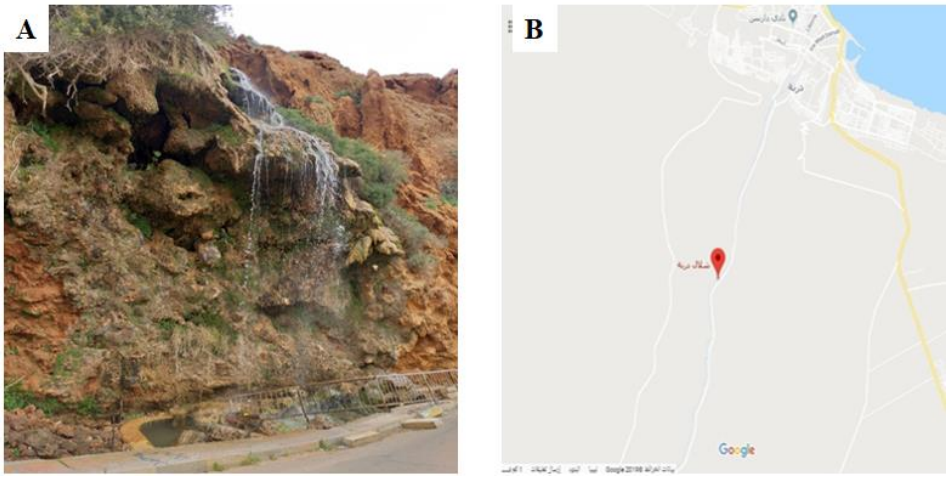

Figure 1. A= A picture of Derna waterfall taken with a Galaxy mobile lens, B= Show Derna waterfall sit, according to Google earth (22.614614, 32.717894).

Figure 2. Map showing the distance between the sampling places along the valley course used in the study

Collection snail's samples

Using forceps and hand nets, 200 snails gathered from nine places around the waterfall; the collection date was from 12 in 2019 to 2 in 2020, and the time of collection of the early morning from 7 clocks to 13 clocks. Snails were collected (Whether alive or dead) from water and mud, as well as from rocks and on plant branches, after that they were transported to the laboratory and washed with soap and water to be cleaned to facilitate identification and classification [16]. After arrival at the laboratory, the snails washed separated according to species, and their numbers recorded, Freshwater snails identified morphologically according to the procedure followed by [17].

Collection fecal samples

Stool samples (approximately 10-15 g) taken at random from female and male residents in the region of the study (ages 4 to 84 years) and put in id-labeled fecal cups, complete data was registered for all, and the samples were sent straight to the lab for analysis. On two separate days, each individual asked to have two fecal samples [18]. Using the Formal and Ether [19].

Collection urine samples

Urine samples from the same individuals were collected in sterile external tubes between 7 and 11 a.m. and were transported to the laboratory, and examined within five hours of collection [20]. 10 ml of urine was aliquot using disposable syringes and filtered through a polycarbonate filter membrane. The filters measured 25 mm in diameter with a pore size of 12 mm; the urine filtered at the sample collection sites. The filtrates mounted on labelled microscope glass slides and deposited in slide boxes for light microscope study [21, 22].

Determination of presence antibodies

This study conducted on 39 people (female and male) living in this area, age 4- 84 years, after getting approval from them. A questionnaire was administered and history taken from them regarding name, sex, age.

10 ml of venous blood from research participants (aged 4 to 84) and arranged it in silica-coated tubes without anticoagulant (vacuum tube). Samples of serum were centrifuged at 12 ℃ for 5 minutes and at a speed of 1500 cycle per minutes (1.5*1000) [23]. Samples were analyzed using Schistosoma IgG ELISA Kit at (Al Razi Laboratory) to perform the immunological analysis using Schistosoma IgG ELISA Kit from DRG Instruments GmbH, Germany up on method described by [24].

RESULTS AND DISCUSSION

This study was aimed to trace the presences different stages of the life cycle of the Schistosoma worm and record it in Derna Waterfall.

Miracidium

Presences of miracidium were conformed at study area which studied under a light microscope (Figure 3 A). Miracidium oval looks conical in the upper body with the mouth opening, as well as a pair of cysts called the Lateral gland on either side, according to the investigation. There are also lengthy tubes termed excretory tubes that stretch to the end of the body in an obvious fashion, as well as cilia all over the body. Miracidium was validated with specifications y reference [18] (Figure 3 B).

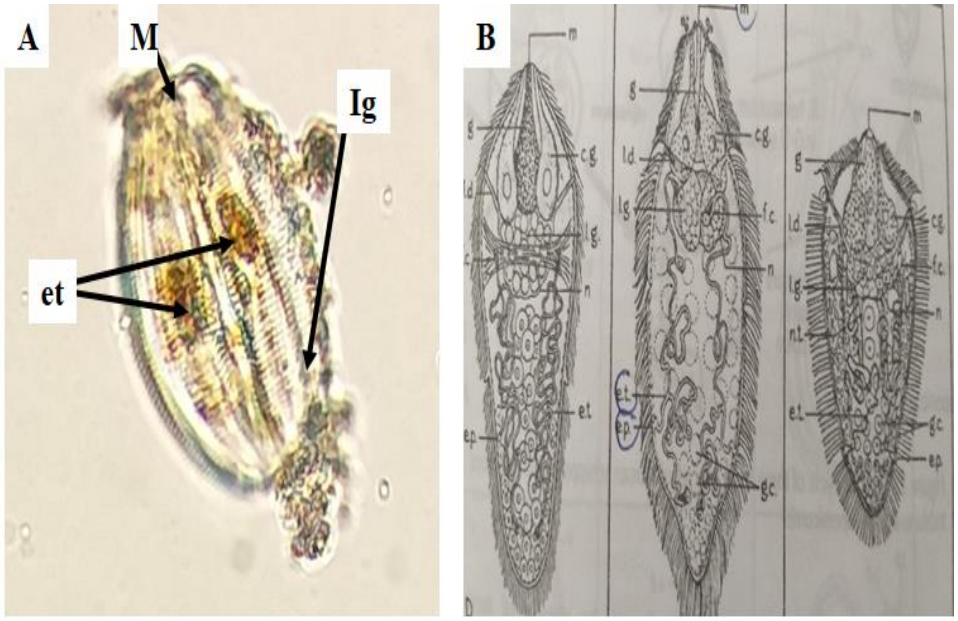

Figure 3. A = The shape of miracidium under a light microscope 10× , B =control miracidium of the Schistosoma of humans ,cg=cephalic glands, e.p =excretory por , e.t=excretory tubule ,f.c=flam cell, g=gut ,g.c=germinal cells, l.d=lateral duct ,l.g=lateral gland ,m=mouth ,n=nervous system , n.t nerve trunk [14]

Eggs

S. haematobium: According to the examination of different samples in the current study, the egg S. haematobium was found in samples that collected. The egg S. haematobium are oval, large and long and bear a conspicuous terminal spine (Figure 4 A and B), Specifications confirmed by reference [25] (Figure 4, C).

S. mansoni: Eggs are large, long and have a characteristic shape, with a prominent lateral spine near the posterior end. The anterior end is tapered and slightly curved. (Figure 5, A). Specifications confirmed by reference [3] (Figure 5, B).

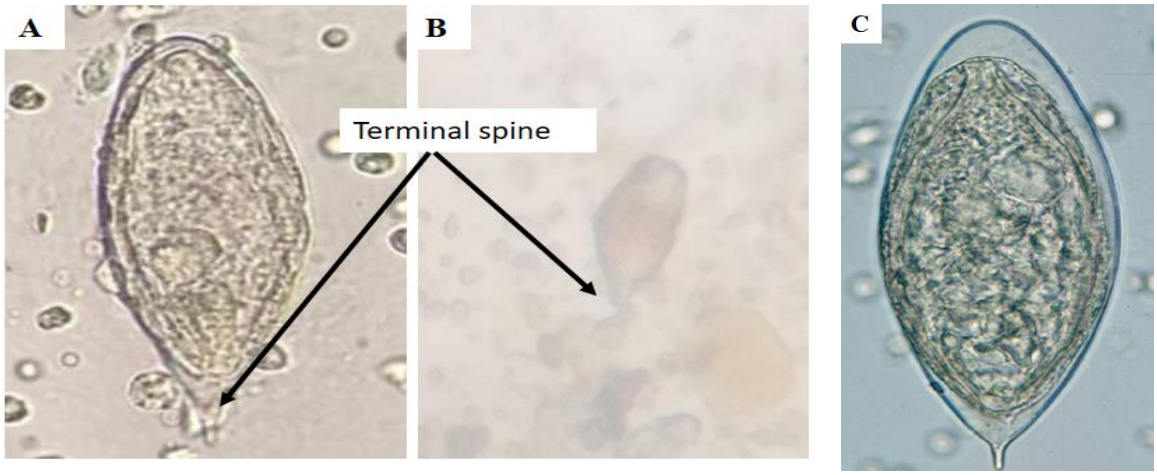

Figure 4. A=Schistosoma haematobium under light microscopy 40× , B = Schistosoma haematobium under light microscopy 10× , C= Control S.haematobium.

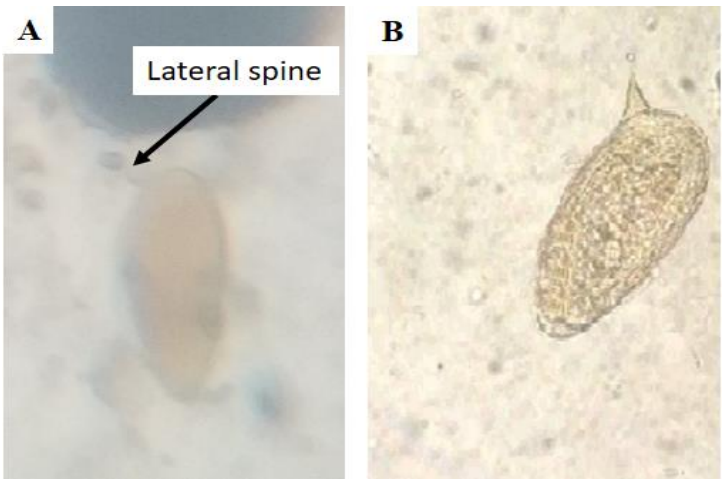

Figure 5. A= S. mansoni under light microscopy10×, B=control, B=control, S.mansoni .

Snails

200 hundred samples were collected from nine places at study area identified by using morphological properties dependent on the shape, size and comparison with[26,27, 28,29]. The following species of snails were determined.

Biomphalaria spp

The shell is thin, and sometimes fragile. It has spirally coiled whorls that rounded at both sides; the shell sculpture is smooth in texture and includes slightly curved regular growth lines, clearly wider than high. The umbilicus is open and wide (Figure 6, A) Specifications were confirmed by reference [30](Figure 6, B).

Bulins spp

From morphological study, the whorls are generally convex and rounded at the periphery and separated by deep sutures. The spire is clearly shorter than the aperture; the umbilicus is visible and varies from small to rather big (Figure 7, A) Specifications confirmed by reference [31] (Figure 7, B). Table 1 was shown percentage number of two species of snails at nine places in study area

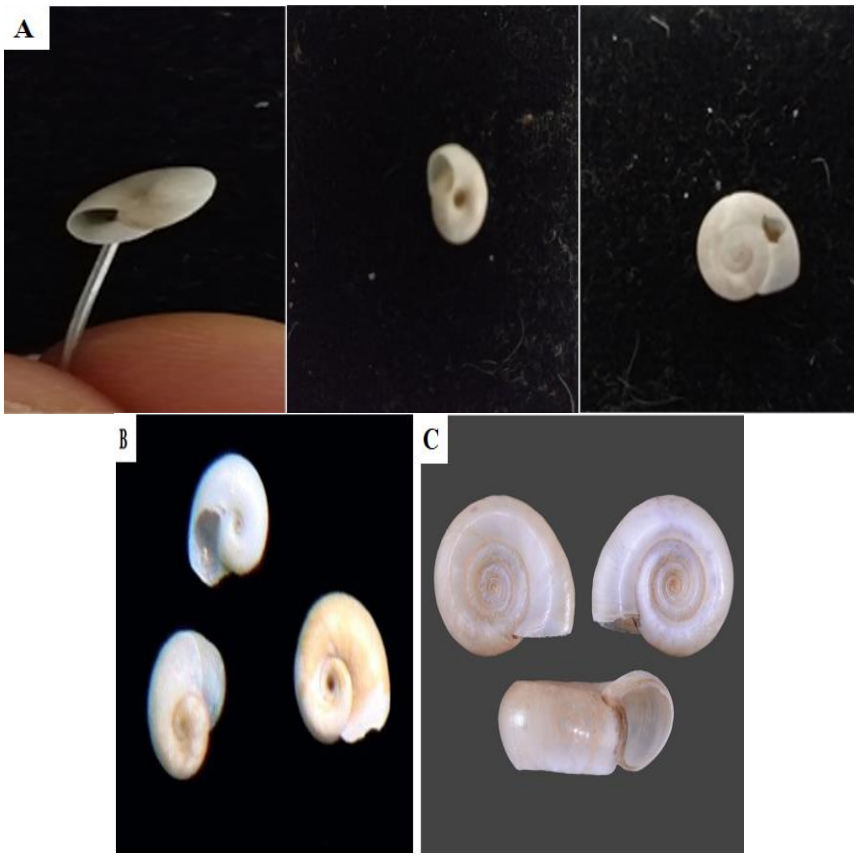

Figure 6. A= Photo of Biomphalaria taken with a Galaxy mobile camera lens, B,C= Control Biomphalaria .

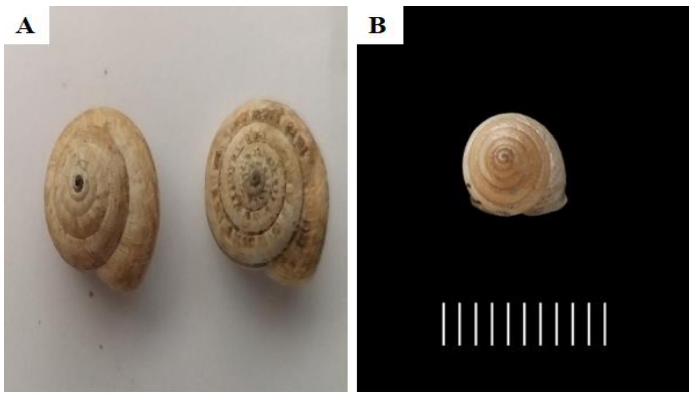

Figure 7. A =Photo of Bulins taken with a Galaxy mobile camera lens , B=Control Bulins .

Table 1. Percentage number of two species of snails at nine places in study area

No. of Place	*Bulins No. (%)*	*Biomphiliaria No. (%)*
1	30 (16.5)	4 (22.2)
2	40 (22)	2 (11.1)
3	12 (6.6)	1 (5.6)
4	10 (5.5)	4 (22.2)
5	13 (7.1)	3 (16.7)
6	20 (11)	0 (0)
7	35 (19.2)	2 (11.1)
8	10 (5.5)	0 (0)
9	12 (6.6)	2 (11.1)
Total	182	18

Immunological analysis results

Immunoassay used for two important purposes: To confirm the presence of infections in humans, and this indicates the adult stages are present, and to support the results of our research. Out of the 39 samples, the incidence of presences of immunoglobulin G (IgG) was according to the use immune kit (Schistosoma IgG ELISA Kit) with percentage value 58.97. Age distribution of total subjects was illustrated in Table 2 with number of positive sample in each category.

Table 2: Presence of IgG antibody in samples of serum at different age in male and female subjects from residence people.

Age(Year)	Male	No.(%)of positive case	Female	No.(%)of positive case
≥15	6	2 (6.66)	2	1 (11.11)
16-20	3	3 (10)	-	-
21-25	2	1 (3.33)	1	1 (11.11)
26-30	1	1 (3.33)	2	1 (11.11)
31-35	6	3 (10)	-	-
36-40	-	-	-	-
41-45	5	4 (13.33)	1	-
46-50	1	-	1	1 (11.11)
51-55	-	-	-	-
56-60	1	1 (3.33)	-	-
61-65	4	4 (13.33)	-	-
66-70	-	-	-	-
≤ 71	1	-	2	-
Total	30	19 (63.31)	9	4 (44.44)

CONCLUSION

Through study and comparison with research in other countries, it has proven that all the phases of the life cycle of *Schistosoma* exist at area of study. Meanwhile, presences of antibody (IgG) in serum of residence at study area were confirmed.

Conflict of Interest

There are no financial, personal, or professional conflicts of interest to declare.

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توطين بعض أطوار يرقات بكتيريا البلهارسيا في شلال درنة، ليبيا

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المستخلص

الخلفية والأهداف. البلهارسيا، وهي إحدى أنواع الديدان المريمية، مسؤولة عن ما يقرب من 80 ألف حالة وفاة سنويًا. وتسبب هذه الديدان مرض البلهارسيا، وهو المرض الطفيلي الأكثر شيوعاً حول العالم. وتبين أن الدراسات حول هذا الموضوع في ليبيا قليلة جدًا، وهي غائبة عمليًا عن موقع هذه الدراسة. وبالإضافة إلى ذلك، فإن قلة الوعي كانت سبباً وجيهاً لدراسة هذا الموضوع؛ هدفت هذه الدراسة إلى تتبع مراحل دورة حياة دودة البلهارسيا وتسجيلها في شلال درنة. **طُرق الدراسة**. تم إخضاع عينات عشوائية من الماء والطين من مياه الشلال للفحص المباشر بالمجهر الضوئي للبحث عن مرحلة الميراسيديوم. وفي الوقت نفسه، تم جمع عينات من القواقع على ضفاف الشلال وتصنيفها في محاولة لتحديد موقع المضيف الوسيط. تم جمع عينات البراز والبول من الأشخاص الذين يعيشون حول ضفة الشلال للبحث عن البيض، وتم تسجيلها. تم جمع عينات الدم من الأشخاص الذين يعيشون حول ضفة الشلال واختبارها باستخدام اختبار ELISA البلهارسيا IgG لتحديد ما إذا كان لديهم أجسام مضادة ضد البلهارسيا. **النتائج**. تم العثور على مرحلة الميراسيديام والبيض في عينات الماء والطين في منطقة الدراسة مما يدل على وجود الطور البالغ. تؤكد نتائج عينات الحلزون وجود مضيفات وسيطة للبلهارسيا، بما في ذلك البيوففالاريا والبولينز. وفي الوقت نفسه، تم العثور على وجود بيض و IgGإيجابي بين الأشخاص الذين يعيشون في منطقة الدراسة. **الخاتمة**. توصي نتائج هذه الدراسة باتخاذ التدابير اللازمة بما في ذلك توعية السكان والقضاء على القواقع التي تعتبر العائل الوسيط للدودة، وبالتالي القضاء على هذه الديدان.

الكلمات الدالة. البلهارسيا، درنة، الشلال، القواقع، ميراسيديام، البيض، البراز، البول وعينات الدم.