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ORIGINAL STUDY

Laboratory Studies on the Prevalence and Cercarial Rhythms of Trematodes from *Bulinus truncatus* and *Biomphalaria Pfeifferi* Snails from Khartoum State, Sudan

Abdel Aziz M Ahmed[1], Nidal A Ibrahim[1], *Mohamed A Idris[2]

تجارب مختبرية لدراسة نسبة ونظم (تواتر) ذوانب الديدان المثقبة في قواقع البولينص ترنكيتص

الملخص: الهدف: (أ)تحديد نسبة الاصابة الطبيعية في قواقع البولاينص ترنكيتص وبيومفيلاريا فايفري بذوانب الديدان المثقبة . (ب) تحديد خروج ونظم ذوانب الديدان المثقبة. (ج) تحديد الوقت الاكثر خطورة للاصابة بذوانب الديدان المثقبة . **الطريقة:** جمعت عينات القواقع من دوار المهدي بولاية الخرطوم . وتم فرز وتصنيف وحفظ القواقع بمختبر البلهارزيا وتمت تغذيتها بالخس . قمنا بفحص القواقع اسبوعيا ولفترة ستة اسابيع . وحفظنا القواقع المصابة في الظلام . أجرينا دراسة سباحة وجلوس الذوانب المختلفة بالمجهر المُجسِّم. قمنا بدراسة نظم الذوانب المختلفة بفحص ثلاث مجموعات خماسية تحت الاضاءة الفلورنسية من الساعة 7 صباحا الى 7 مساء . و كذلك فحصنا مجموعات متشابهه من الساعة 7 مساء الى الساعة 7 صباحا . **النتائج:** وجدنا 187 (14.9%) ترنكيتص بولاينص مصابة بأربعة أنواع من الذوانب من مجموع 1257 . وسجلت ذوانب البلهارزيا أعلى نسبة (9.5%)، تلتها ذوانب الامفيستوم (2.5%) . والذيفيدوسركاريا (2.4%) وأخيرا ذوانب الطيور (5%). ومن مجموع 200 بايومفيلاريا فايفري وجد ان 22 (11%) مصابة فقط بذوانب الذيفيدوسركاريا . دراسة نظم الذوانب بينت أن ذوانب البلهارزيا بدأت الخروج من الساعة 7 صباحا لتصل ذروتها ما بين الساعة 11صباحا الى الواحدة بعد الظهر. كما أن نظمية ذوانب الطيور كانت متشابهة لنظمية ذوانب البلهارزيا . لكن وصلت ذروتها بين الساعة 9-11 صباحا . أما ذوانب الذيفيدوسيركاريا والامفيستوم بدات اعلي معدلات خروجها الساعة 7 صباحا ثم تناقصت تدريجيا لاعداد قليلة أو توقفت تماما بالتوالي مع غروب الشمس . **الخلاصة:** توفر المعلومات حول سلوك ذوانب الديدان المثقبة ذو فائدة كبيرة لعدم التعرض للاصابة بها . خاصة البلهارزيا . و ذلك عن طريق الابتعاد عن المياه الملوثة أثناء ذروة وجود الذوانب . كما أن دراسة الخروج والنظم قد تساعد في تصنيف الاجناس والانواع المتشابهة لهذه الذوانب .

ABSTRACT ***ObjectiveS:*** (a) To determine the natural infection rate of *Bulinus truncatus* and *Biomphalaria pfeifferi* snails with trematodes' cercariae. (b) To determine the emergence and rhythmicity of cercariae. (c) To elucidate the high-risk time for man and other animals to acquire infection. ***Methods:*** Snails were collected from Dawar El Mahadi Agricultural Scheme, Khartoum State, identified in the laboratory, kept at room temperature and fed on lettuce. The snails were screened weekly for six weeks for natural infection and infected snails were kept in the dark. The swimming patterns and resting position of the freshly emerged cercariae were studied using a stereomicroscope. The rhythmicity of the different types of cercariae was studied by screening three sets of 5 naturally infected snails under fluorescent light from 07.00 to 19.00 and similar sets from 19.00 to 07.00. ***Results:*** Out of 1,257 screened *Bulinus truncatus,* 187 (14.9%) shed four types of cercariae. The highest prevalence of natural infection (9.5%) was by schistosome cercariae followed by amphistome (2.5%), xiphidiocercariae (2.4%) and lastly by avian cercariae (0.5%). However, out of 200 screened *B. pfeifferi*, 22 (11%) shed only xiphidiocercariae. The rhythmicity studies showed that the emergence of schistosome cercariae increased steadily from 07.00 to reach its peak at 11.00-13.00. The emergence rhythms of avian cercariae are similar to those of the schistosome, but with an early peak at 09.00-11.00. The xiphidiocercariae and amphistome cercariae started with high rate of emergence at 07.00. and decreased gradually to very low levels or complete disappearance, respectively, around sunset. ***Conclusion:*** Information on cercarial rhythmicity and chronobiological characteristics are thought to be useful in avoiding water contact during high-risk time of infection and may be helpful in the identification of closely related species and strains of cercariae.

Key words: *Bulinus*, *Biomphalaria*, prevalence, cercarial rhythms, schistosomes, amphistome, avian, xiphidiocercariae.

[1]Schistosomiasis Research Laboratory, Faculty of Science, University of Khartoum, Sudan, [2]Department of Microbiology & Immunology, College of Medicine & Health Sciences, Sultan Qaboos University, P.O. Box 35, Al Khod 123, Muscat, Sultanate of Oman

**To whom correspondence should be addressed. Email: midris@squ.edu.om*

Schistosomes are helminthes of the class Trematoda that alternate generations, with a sexual phase in definitive mammalian hosts and an asexual phase in intermediate snail hosts. In humans, these blood flukes reside in the mesenteric and vesical venules. They have a life span of many years and daily produce large numbers of eggs, which must traverse the gut and bladder tissues on their way to the lumens of excretory organs. To understand the transmission dynamics of the disease, it is necessary to highlight essential segments in the process of acquiring an infection. Thus, knowledge of cercarial infection rates, not only in individual habitats and the area as a whole, but also in relation to seasonal population changes, is crucial.[1,2] Other segments like the susceptibility of the snail to infection and the effects of such infection, the pattern and number of cercarial production and the factors influencing cercariae are of paramount importance in aspects related to the transmission of schistosomiasis.

As relatively few studies were conducted on the production and dynamics of trematodes cercariae in Africa, the main objectives of this study were to determine the prevalence, highlight the cercarial emergence of trematodes from naturally infected snails and to elucidate the high risk time for man and other animals to acquire infection.

METHODS

snail collection

Four field surveys were conducted for systematic snail sampling. A standardized scoop was used for sampling some minor canals in Dawar El Mahadi agricultural scheme, Khartoum State. In each field survey, the collected snails were pooled in plastic bowls and lined by grass cover from the canals. The collected snails were transported to the Schistosomiasis Research Laboratory, University of Khartoum, where different snail species were isolated, cleaned and a series of laboratory experiments were conducted.

prevalence of natural infection

The collected snails were screened weekly, for six weeks, for the presence of trematodes natural infection. Patches of (20-30) cleaned snails were put in small glass containers containing about 20 ml of warm water (30-38°C) and placed under artificial light from 10:00 to 13:00 to enable cercarial emergence. The glass containers showing cercariae were identified and the snails were screened individually for cercarial shedding. Snails (*Bulinus* and *Biomphalaria*) observed to shed any trematode cercariae were placed in separate plastic bowls containing 5 liters of dechlorinated water and kept in the dark at room temperature (30-35°C).

observation of living unstained cercariae

Immediately after cercarial shedding the snails that liberated the same type of cercaria were placed in the same plastic bowls. The different freshly emerged cercarial specimens were placed, separately, in a water volume sufficient to allow their normal behavioural pattern. The swimming patterns and resting position of the cercariae were observed using a stereomicroscope of 8-40 times of magnification. To observe the cercarial developmental pattern, a piece of cellophane was introduced to enable cercarial encystation on external substrate (if any). The identification of cercariae to the major type level was carried out by transferring living and unstained cercariae onto a glass slide. A cover slip was added and excess water was drawn off by applying a strip of filter paper to one side of the cover slip.

staining and identification of cercariae

The different types of cercariae were collected from naturally infected snails by emergence then prepared in permanent slides using Ehrlich's Haematoxylin and were identified following Frandsen and Christensen.[3] It was observed that the morphological features in the stained cercariae are more easily observable than in unstained ones. The diagnostic characteristics used in the identification of the cercariae included: general appearance, tegument, body suckers, alimentary system, excretory system, gland cells, genital primordium and cercarial tail. Four types of cercariae (human schistosome, xiphidiocercaria, amphistome and avian) were identified.

cercarial emergence and rythmicity

Of each cercarial type, three sets of five naturally infected snails were placed in small beakers each with 15 ml of warm water (34-40°C) and placed under fluorescent light from 07:00 to 19:00. The water with cercariae was changed in concurrent sets every two hours. Three samples, 50 micro litres each, of the cercarial suspension in the beaker, were taken by a Finn pipette into small Petri dishes. Drops of iodine were added to kill and stain the cercariae, which were then counted

Table 1: ***Means and ranges of cercarial emergence from naturally infected B. truncatus (snail/day)***

Type of cercariae	Means and ranges
Schistosome cercariae	135 (53 - 252)
Xiphidiocercariae	827 (198 - 2430)
Amphistome cercariae	82 (14 - 106)
Avian cercariae	285 (274 - 291)

under a dissecting microscope. Averages of the cercarial numbers in the 3 samples were calculated. Then the total numbers of cercariae in the suspension were calculated as follow

The mean number of cercariae in 50 microlitres X 20 X volume of suspension in millilitres. Other similar sets, replications and calculations were carried for cercarial emergence from 19:00 to 07:00.

statistical analysis

Based on the distribution of the observation, paired t-test and Wilcoxon matched pairs, signed ranks tests were used to determine the level of significance in the differences of related samples of paired observations. In addition, all normally distributed related samples of more than two observations were treated with Friedman twoways ANOVA.

RESULTS

prevalence of natural infection

Out of 1,257 screened *B. truncatus* snails, 187 (14.9%) shed four different types of cercariae. The highest prevalence of natural infection was shown by schistosome cercariae (9.5%) followed by amphistome (2.5%), xiphidiocercariae (2.4%) and lastly by avian cercariae (0.5%). The observed differences in the snails' infection rates were found to be statistically significant ($p < 0.05$). Out of 200 *B. pfeifferi* screened snails, 22 (11%) liberated only xiphidiocercariae.

cercarial production

Table 1 shows the means and the ranges of cercarial production from naturally infected *B. truncatus* snails. The number of xiphidiocercariae liberated daily was more than 62% of the total cercariae. At the very commencement point, xiphidiocercariae were more than twice the total number of other emerging cercariae, as shown in Table 2. Amphistome cercariae were the least observed in both aforesaid parameters. Statistical analysis revealed high significant differences ($P < 0.001$) in the rate of different cercarial emergence. Further analysis, via Scheffe test, reassured that such differences were mainly attributed to xiphidiocercariae.

innate rhythms

Tables 2 summarises the patterns of innate diurnal rhythms of the four types of cercariae, liberated from naturally infected *B. truncatus*. The individual types of cercariae showed a remarkable constant daily periodicity, though day-to-day irregularity in numbers of cercariae shed was observed. In the naturally infected snails, cercariae emergence began at sunrise, around 07:00. In schistosome cercariae, the emergence increased to reach a peak at 11:00-13:00 and gradually decreased to disappear around sunset at 19:00. The avian and xiphidiocercariae were like the schistosome in the general pattern, but with an early peak at 09:00-11:00. However, avian cercariae showed substantial nocturnal emergence. On the other hand, the amphistome cercariae started from the highest rate of emergence at sunrise to sharp decrease at early afternoon and to complete disappearance around sunset.

DISCUSSION

The full understanding of the transmission pressure of a trematode infection in a given area must include knowledge of cercarial production and dynamics. The present study emphasized that the local strain of *B. truncatus* is susceptible to the local *S. haematobium* strain. Also, to the best of our knowledge a single study was conducted more than 20 years ago in North Gezira, Sudan and gave 3.3% patent infection of *B. truncatus* by *S. haematobium*[4] which is much lower than the 9.5% reported in this study. The reported natural infection (0.2%) of *B. pfeifferi* snails by *S. mansoni* in the Sudan was by far less,[5,6] but comparable to the prevalence of natural infection (7.9%),[7] in *B. arabica,* a strain of *B. pfeifferi*,[8] from the Sultanate of Oman.

Cercarial emergence is the final product of many biotic and abiotic factors.[9] Productivity also depends on the host-parasite compatibility.[10] If a strain of snail is very receptive, the parasitic process will be longer and the cercarial productivity greater.[11,12] The maximum mortality rate was observed among snails with high natural infection, immediately after the first trial for cercarial shedding. The intensity of the snails' natural infection was expressed in their multiple infections as well as the high frequent shedding of cercariae. Such a mortality rate was expected, since infected snails are comparatively more sensitive to environ-

Table 2: ***Emergence rhythms of cercariae from naturally infected B. truncatus***

Time of day(Hours)	Number and types of cercariae			
	schistosome cercariae	Xiphidio-cercariae	Amphistome cercariae	Avian cercariae
07:00 - 09:00	140 ± 24	1430 ± 51	264 ± 33	293 ± 29
09:00 - 11:00	160 ± 9	1849 ± 108	118 ± 26	570 ± 32
11:00 - 01:00	220 ± 43	421 ± 84	21 ± 3	201 ± 26
13:00 - 15:00	140 ± 11	236 ± 47	6 ± 2	163 ± 9
15:00 - 17:00	12 ± 3	115 ± 23	2 ± 0.3	110 ± 6
17:00 - 19:00	2 ± 0.5	86 ± 17	0.0	90 ± 8
19:00 - 07:00	60 ± 3	2.0 ± 0.3	230 ±24	2480 ± 92

mental changes than non-infected snails. It seems that the temperature and the water quality are the factors behind the increased death rates among the naturally infected snails.[12] The longevity of infected snails in the field is determined by different factors such as: age of the snail, super-infection and effect of schistosome miracidia and other parasites. However, the lifespan of the naturally infected snails could not be elucidated in the field, since the date on which the snails became infected was not known.[9]

Studies on trematode emergence in freshwater systems suggest that the larval emergence from snails varies according to the circadian cycle and is optimized to enhance the probability of successful transmission.[13-17] The present study proved that the cercarial shedding pattern of *S. haematobium,* although it peaked early (11:00-13:00), does not differ from those reported by other workers. Cercariae of *S. mansoni* in the Gunaid scheme peaked 13:00-15:00,[3,9] while the same species in the Gezira scheme peaked 12:00-14:00.[1] It seems that the peak of the *S. haematobium* cercarial rhythm coincides with the peak of human water-contact activities during the day. The cercariae aves are like schistosomes in this manner, but with an earlier peak, 09:00-11:00, moving to a complete disappearance around dusk time.

The cercarial emergence rhythm is closely correlated with periods of activity of the most permissive host. Human schistosomes have a diurnal emergence rhythm. *S. rodhaini*, which infects wild rodents, has a nocturnal emergence. The ultradian emergence rhythm of *S. margrebowiei*, which peaks at dawn and dusk, is thus perfectly adapted to antelopes coming to water pools early in the morning and late in the evening. This variability of emergence rhythms, documented at the interspecific level, was recently observed at intraspecific level by the discovery of chronobiological polymorphism in populations of *S. mansoni* from a single endemic area.[18] In Sudan, the schistosome cercarial emergence peaks vary over an interval of twelve hours from one scheme to another, between 6:00 to 18:00, depending on the *Schistosoma* population considered.[1]

Findings on cercarial emergence rhythmicity might reveal micro-evolutionary shifts taking place in schistosome populations. Obviously, data on cercarial chronobiological characteristics may well prove of value for the identification of closely related species and strains.[8] Thus, thorough systematic observations are crucial to elucidate these characteristics. Furthermore, such studies might explain the role of different factors affecting the cercarial rhythmicity and production dynamics, especially in the field.

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