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Factors Affecting Dynamics of Metacercarial Productivity of *Fasciola gigantica* from its Snail Host

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Abstract: Experimental infections of preadult and adult *Lymnaea cailliaudi* snails using the same isolates of *Fasciola gigantica* miracidia were performed under laboratory conditions to determine whether the temperature, intensity of infection and age of snail host of *F. gigantica* in Egypt had an effect on the dynamics of metacercarial productivity. Preadult snails were divided into 3 groups kept at 18-20, 24-26 and 29-31°C, respectively and the adult snails were represented by a single group kept at 24-26°C to compared to its corresponding preadults ones. Each group was divided into four subgroups a, b, c and d, each one was subjected to 1, 2, 3 and 5 miracidial exposures, respectively. Two miracidial infections per snail produced more metacercariae compared to that of 1, 3 or 5 miracidial infections. Prepatent period of *F. gigantica* inside its snail host was inversely related to temperature and markedly affected by age rather than the number of miracidia inoculated. In contrary, Patent periods were significantly affected by the intensity of miracidial infection. Temperature above 24°C was suitable for high metacercarial production and a significant increasing in the percentage of the floating metacercariae was found at high temperature. Adult snails showed less susceptibility to infection as they never infected with single miracidia and produced few metacercariae with more than single miracidial infection.

Key words: *Fasciola gigantica*, *Lymnaea* spp. age class, intensity of infection, temperature, metacercarial output

INTRODUCTION

Fascioliasis is an increasingly recognized public health problem in Egypt for both animal and human in the last few years[1,2]. However, *Lymnaea* species, as intermediate hosts of *Fasciola gigantica* or *Fasciola hepatica*, have a crucial role in completion of the life cycle and the transmission of the parasite to animals and man[2-4]. Many works have been published on the development of *Fasciola sp.* insides its snail host[5-10] however few studies have been made on the dynamics of cercarial emergence and production from their snail hosts which is important to better understand the epidemiology of Fascioliasis. Hence, for supporting the epidemiological studies required for control of fascioliasis, the present work aimed to study the possible influence of some factors including temperature, snail age and number of *F. gigantica* miracidia inoculated to *L. cailliaudi* snails on the dynamics of emergence and production of metacercariae.

MATERIALS AND METHODS

***Lymnaea cailliaudi* culture:** *L. cailliaudi* snails (synonymous: *L. natalensis*), the intermediate host of *F. gigantica* in Egypt, were collected from water bodies in different villages of Ismailia governorate and were reared and bred through several generations in the laboratory. The snails were kept in plastic containers (20 x15 and 7 cm deep). A conditioned tap water was used with a continuous aeration. The aquaria were placed in a light-dark cycle of 10 h of light from 7 a.m. to 5 p.m. Aquatic plants such as "Elodea" were required for the snails for deposition of their egg masses[11]. Snails were fed on dried lettuce three times per week. Newly hatched snails would not eat dried lettuce, so they were fed on dried fishmeal three times per week for rapid growth.

Experimental infection of snails with *F. gigantica* miracidia: Mature *F. gigantica* flukes were collected from the bile ducts of cattle slaughtered at the slaughterhouse

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of Ismailia City. *F. gigantica* eggs were obtained from the uterus of the flukes. The eggs were washed several times with distilled water and then incubated in Perti-dishes containing water in complete darkness at ambient temperature[12]. The hatching of the eggs into free miracidia was induced by exposed the petri-dishes to strong light.

Immature snails (shell length: 3 mm and age: 35-45 days) of the laboratory cultured generation were selected from the aquaria. The collected snails were divided into 3 groups I, II and III and kept at 18-20, 24-26 and 29-31°C, respectively. Each group was divided into four subgroups a, b, c and d each was infected with 1, 2, 3 and 5 miracidia, respectively. Control groups of uninfected snails were also selected for each experiment. Adult snails of laboratory cultured generation, group IV were selected (shell length: 7 mm and age: 100-110 days) and divided into 4 subgroups a, b, c and d and which were infected with 1, 2, 3 and 5 miracidia, respectively and kept at 24-26°C. The snails were put individually in wells of microplates with 3 ml dechlorinated water. Active miracidia were collected within 1 h after hatching and picked up with a pipette under the dissecting microscope and then introduced into the microplates wells, each snail was kept in the wells containing miracidia for 4 h. Penetration of miracidia into the snails was observed under dissecting microscope. After exposure to miracidia, all the infected snails were maintained in the standard aquarium condition described before, each subgroup in separate labeled aquaria.

The snails of each subgroup were examined under binocular dissecting microscope through their shell every 5 days for observation of the development of *F. gigantica* larvae. Before cercarial shedding, each snail was maintained in Petri dish (10 cm). Fresh distilled water was added daily for enhancement of cercarial shedding[6]. The first day of cercarial shedding was detected for all groups where the shed cercariae were allowed to be fixed on either cellophane sheets or on the wall of the dishes[13], other cercariae were encysted on water surface (floating cysts).

Parameters considered: During the time from miracidial exposure to the time of the snail's death, the following parameters were considered: Infection and mortality rates of *L. cailliaudi* snails during the prepatent period, duration of prepatent and patent periods, total number of metacercariae (fixed and floating metacercariae, free cysts and the dead cercariae) which were counted daily at fixed clock under the dissecting microscope and the number of the snails that shed cercariae and those which unshed cercariae.

Statistical analysis: The mean (±SD) was calculated for all the individual data collected from each subgroup in this study. Statistical analysis was performed using one way analysis of variance (ANOVA) for assessment of the statistical Significance of difference. All the statistical tests were performed using computer software (Excel).

RESULTS

Infection and mortality rates: Infection rates in the entire snail groups with *F. gigantica* miracidia are shown in Table 1. Infection rates of preadult snails were markedly affected by rising in temperature, the mean (±SD) overall infection rate was highest in group II and I, (80.75±21.9) and (80.5±20.4%), respectively, followed by group III (66.75±25.4%), ($P<0.001$). On the other hand, infection rates with single miracidium were very low compared to rates recorded with more than one miracidial infection in all groups ($P<0.001$). Snails' age had a significant effect on the susceptibility to infection as adult snails did not infect with single miracidium, (group IVa). The mean (±SD) overall infection rate of group IV was (52±2%) which was significantly lower compared to overall rate of the corresponding preadult snails (group II) kept at the same temperature ($P<0.001$).

Regarding to study the effect of temperature on mortality rates of snails during prepatent period Table 1, the mean (±SD) overall morality rates were (32.2±4.8%) in group I, (27.9±4.2%) in group II and (29±3.9%) in group III, no significant differences were detected. Lowest mortality rates were recorded in two miracidial infection, while the highest one was recorded in 5 miracidial infection. It is worth mentioning that, in one miracidial infection the mortality rates were higher than in 2 or 3 miracidial infection and approached to the rates of 5-miracidial infection in some groups. The overall mortality rate was (25.3±4.3%) in the adult group (IV), which was not significantly different from the overall mean of its corresponding group II.

Prepatent period: The prepatent periods of *F. gigantica* larvae inside the snails groups (I-III) are shown in Table 1. The duration of the prepatent period of *F. gigantica* inside its snail host was inversely related to temperature. A rolonged prepatent periods (99.9±0.8 day) were recorded for group I and this period was shortened to (73.3±1.1 and 37.3±0.81 day) for groups II and III, respectively ($P<0.001$). Although there were differences in the first day of cercarial shedding among all the subgroups, these differences were not significant. The mean (±SD) days of the longest prepatent period was (100.2±3.7) in group (Ia), while the shortest period was

Table 1: Mean (±SD) infection and mortality rates of *L. caillicaudi* snails infected with *F. gigantica* miracidia and Mean (±SD) duration of prepatent and patent periods (days) of *F. gigantica* inside its snail host

Patent period (days)	Prepatent period (days)	% Mortality	% Infection rate (Number of snails)		Group
4.4±1.1	100.2±3.7	32.17±7.81	50(10.0±1.41)	a	I (18-20°C)
11.2±1.6	98.6±2.7	26.91±3.68	91(18.2±1.1)	b	
9.0±1.0	100.4±4.0	31.68±3.68	93(18.6±0.55)	c	
7.0±1.2	100.2±3.0	38.58±2.93	88(17.6±1.34)	d	
7.8±2.8	72.0±3.1	27.31±9.37	48(9.6±2.7)	a	II (24-26°C)
13.0±4.3	73.2±3.3	23.08±7.06	93(18.6±1.14)	b	
11.2±2.9	73.2±3.0	27.94±8.28	89(17.8±0.84)	c	
9.0±2.6	74.6±4.7	33.25±5.04	93(18.6±0.9)	d	
5.2±2.8	36.4±2.4	27.05±8.46	30(6.0±1.58)	a	III (29-31°C)
7.0±4.2	37.4±2.7	26.94±5.48	70(14.0±1.87)	b	
6.2±1.3	37.0±3.3	27.29±7.23	81(16.2±1.14)	c	
5.2±2.4	38.4±3.0	34.88±5.47	86(17.2±1.48)	d	
0.0	0.0	0.0	0	a	IV (24-26°C)
9.4±2.3	80.0±3.39	22.47±15.88	50(5.0±1.58)	b	
7.0±1.87	79.0±5.15	23.05±18.29	54(5.4±1.52)	c	
7.0±1.87	80.2±4.82	30.24±9.87	52(5.2±1.64)	d	

Table 2: Mean (±SD) number of Cercarial Output from *L. caillicaudi* snails infected with *F. gigantica* miracidia and Mean (±SD) % of snail which shed and not shed cercariae

		Cercarial output						
% Cercarial unshed snails	% Cercarial shed. snails	Total cer.	Dead cer.	Free cysts	Floating metacer	Fixed metacer		Groups
29.55±9.49	70.45±9.49	54.4±24	0.2±0.5	0.0	11.8±6.30	42.4±18.7	a	I (18-20°C)
29.34±4.16	70.66±4.16	199.8±74	0.2±0.5	0.2±0.50	37.4±17.3	162.0±59.2	b	
32.37±7.16	67.63±7.16	136.8±76	0.4±0.5	0.0	28.8±17.6	107.6±58.9	c	
31.58±5.33	68.42±5.33	115.6±55	0.2±0.5	0.0	22.4±5.30	93.0±51.0	d	
32.50±7.02	67.50±5.01	186.0±78	0.6±0.5	0.4±0.50	44.6±19.7	140.4±63.0	a	II (24-26°C)
28.53±3.64	71.47±3.64	580.0±18	1.2±0.5	0.4±0.50	121.8±45.1	456.6±136	b	
31.28±4.81	68.72±4.81	333.4±19	0.6±0.9	0.4±0.50	75.8±55.7	256.6±145	c	
30.58±5.85	69.42±5.85	219.8±75	0.6±0.5	0.2±0.50	49.4±12.6	169.6±63.6	d	
32.67±5.33	67.33±5.33	121.8±62	2.2±1.9	0.8±0.80	48.0±29.1	70.8±33.8	a	III (29-31°C)
29.44±4.29	70.57±4.29	277.8±12	2.4±1.1	1.2±0.50	91.6±39.7	182.6±82.0	b	
34.42±9.92	65.58±9.92	198.0±84	1.8±0.8	0.4±0.50	62.8±32.7	133.0±52.5	c	
29.92±9.50	70.08±9.50	161.4±81	2.6±1.7	0.4±0.50	53.8±24.7	104.6±56.5	d	
0.0	0.0	0.0	0.0	0.0	0.0	0.0	a	IV (24-26°C)
28.33±7.45	71.670±7.45	185.8±75	1.8±1.5	1.2±0.80	43.0±16.7	139.8±61.6	b	
30.33±7.85	69.670±7.85	106.4±35	1.4±1.8	1.6±1.14	24.8±8.64	78.6±28.13	c	
29.67±18.94	70.33±18.94	75.0±34	0.6±0.9	1.8±0.84	15.0±8.5	57.6±25.6	d	

(36.4±2.4) in group (IIIa). The effect of snail age on the duration of prepatent period (Table 1), showed that the mean (±SD) overall prepatent period was significantly longer in group IV (79.73±0.6) compared to its corresponding group II (73.25±1.1); ($P<0.001$).

Patent period: The patent periods of *F. gigantica larvae* in all groups are shown in Table 1. The means (±SD) of the overall patent periods were 7.9±2.9, 10.25±2.31 and 5.9±0.87 for group I, II and III, respectively, a significant effect of temperature on duration of patent period of *F. gigantica* was recorded only at high temperature 29-3°C, where the duration of the patent period was shorter than those recorded at 24-26°C ($P<0.01$). On the other hand, patent period was not markedly affected by temperature rather than it was affected by the variation in number of inoculated miracidia. In group I or II significant differences were found between subgroups (a) infected with single miracidium and subgroups (b) or (c) infected with 2 and 3 miracidia, respectively ($P<0.001$). The means (±SD) days of the patent period in groups Ia and IIa (4.4±1.1 and 7.8±2.8, respectively) were significantly lower compared to means recorded for groups Ib and IIb (11.2±1.6 and 13±4.3, respectively) and for groups Ic and IIc (9±1.0 and 11.2±2.9, respectively). The longest patent period was recorded in group IIb (13±4.3), while the shortest period was recorded in group Ia (4.4±1.1). Table 1 shows that, mean (±SD) patent period of the adult snail group IVb (9.4±2.3) was significantly lower compared to group IIb (13±4.3); ($P<0.05$). However, no differences between subgroups c or d of group IV and their corresponding ones in groups II were found.

Cercarial output: Table 2 groups the mean (±SD) number of cercariae shed per snail in groups (I-III), Mean values were ranged from (54.4-199.8) in group I, (186-580) in group II, (121.8-277.8) in group III. The overall metacercarial production was significantly influenced by the temperature, the mean was highest in group II (329.8±154.43) kept at 24-26°C followed by group III

(189.75±66.45) kept at 29-32°C while the lowest mean was (126.65±59.99) in group I kept at 18-20°C. In studying the effect of intensity of infection on cercarial productivity, the mean total number of cercariae shed in the three groups was highest in subgroups (b), (352.53), followed in decreasing order by subgroups (c), (222.73), (d), (165.6) and (a), (120.73). Snails of subgroups (b) produced more metacercariae than groups a, c and d ($p<0.001$), no significant differences between subgroups (a) and (d) were found. The highest mean (±SD) of the total number of cercariae shed per snail was recorded in group IIb (580±181.6), while the lowest mean was recorded in experiment Ia (45.4±24.8).

Table 2 shows the mean number of the different types of cercariae shed from the snails in each subgroup. Some cercariae died after their exit from the snail but their number is very low (0.6 to 2.6%) in group I, II and III. The number of the dead cercariae was significantly higher in group III compared to group I and II ($P<0.01$). Other cercariae fixed on support or turned into floating cysts. The percentage of floating cysts was significantly high in group III (34.3%) followed by (22.55%) in group II and (20.21%) in group I ($P <0.001$).

Mean (±SD) number of cercariae shed in adult group IV (Table 2) was significantly high in subgroup (b), (185.8±75.44) followed by subgroup (c), (106.4±35.15) and (d), (75±34.31); ($P < 0.01$). The mean number of cercariae shed in subgroups b, c or d was highly significantly lower when compared to their corresponding subgroups of group II ($P < 0.001$). The mean (±SD) overall cercariae shed in group IV was significantly low (122.4±57.11) compared to mean group II (329.8±154.43); ($P<0.001$).

Some of the infected snails died without shedding of cercariae in all experimental groups. The mean (±SD) percentages of snails, which shed cercariae and those, which did not shed cercariae, are shown in Table 2. In group I, II, III and IV, the mean values of cercarial shedding snails were ranged from 65.58 to 71.67% and for unshedding ones from 34.42 to 28.33%. There was no significant influence with the number of inoculated miracidia or the temperature or age of snails on the percentage of snails that shed cercariae although differences were found. In addition, the percentage of snails which shed cercariae, was significantly higher than snails which unshed cercariae ($P<0.001$).

DISCUSSION

Intramolluscan-trematode dynamics can be affected many factors including a variety of forces, both internal and external to the snail which affects the manner in which the larval development occurs[28]. Accordingly, our study attempted to determine the influence of some factors (Temperature, intensity of infection and age of the snail) on the emergence and productivity of *F. gigantica* cercariae.

Infection rates of *L. cailliaudi* snails with *F. gigantica* miracidia in this study were markedly affected by temperature, intensity of infection and age of the snails. Low infection rates at high temperature may be attributed to the effect of temperature on the activity of miracidia as the length of *F. gigantica miracidia* life span was inversely related to the temperature degree[9]. Smith and Grenfell[14] found that the mean expected life span of the miracidium decreases from about 35 h at 6°C to about 6 h at 25°C. Moreover, Infection rates with single miracidium were very low compared to rates of more than one miracidial infection. In this regard, Shahlapour *et al.*[4] found that the infection rates in *L. peregra* (40-50 day) infected with 1 and 2 *F. gigantica* miracidia was 33.3 and 86.6%, respectively and the rates in adult and (1-3 weeks) *L. staginalis* snails infected with 4 miracidia were 0 and 75.8%, respectively. This indicated that the snail age might effect on the susceptibility to infection with *F. gigantica* miracidia which in turn supported our findings. Smith and Grenfell[15] found that the probability of a snail remaining uninfected after exposure to a single miracidium varies with the size class of the snail. The probability was 0.46-0.48, 0.27-0.26 and 0.13-0.19% for snail size classes 0.5-2.9, 3.0-5.9 and 6.0-8.9 mm, respectively under the conditions of the experiment temperature 15°C, exposure period 30 min, water volume 8 ml.

Our results showed that the duration of the prepatent period of *F. gigantica* inside its snail host was inversely related to temperature. In warm season in East Africa, 75 days are required for the development of *F. gigantica* in the snail, this being extended to 175 days in the cold season[16]. The prolonged prepatent period was explained by Wilson and Draskau[17] and El-Bahy[9] who found that, the first rediae produced from the sporocyst under the winter condition continued to produce successive redial generation and the cercariae appeared inside the developed rediae with the gradual rise of temperature. The prepatent period was about 75 days in *L. truncatula* infected with single *F. gigantica* miracidium at 23°C [18], 70 days in *L. auricularia* infected with two *F. gigantica* miracidia at 24°C[4] and 75 to 85 days in *L. natalensis* infected with *F. gigantica* miracidia at 26°C[8]. These results were nearly in agreement with our result if the maintenance temperature, number of miracidia and snail species were taken into consideration.

Our study showed also that, intensity of infection at exposure does not so much affect the delay in cercarial

emergence and there was a relation between snail age and the delay in cercarial shedding as longer prepatent period was recorded in adult snails. Our findings were in agreement with results of Hodasi[7], Itagaki and Itagaki[19] and Lee *et al.*[20]. In contrary, the results of Shahlapour *et al.*[4] showed a significant difference in the duration of the prepatent period in *L. peregra* infected with single and two *F. gigantica* miracidia, 70 and 57 days, respectively.

Concerning the patent periods recorded in this study, Lee *et al.*[20] found that 4.5 mm *L. viridis* kept at 20-24°C and infected with three *F. hepatica* miracidia recorded a long patent period (26.8±7.5 days) than snails infected with one (18.3±4.2 days) or five miracidia (20.5±0.7 days). At 20°C, the patent period of 4 mm *L. trunactula* infected with two *F. gigantica* miracidia was ranged from 17.3 to 23.8 days according to population[21]. However, the duration of patent period recorded by Itagaki and Itagaki[19] was extended over 15 weeks, this duration is exceeding the present result. Moreover, the age of the snail had a significant effect on the duration of the patent period, patent period of *Fasciola* in adult *Lymnaea* snails was significantly shorter than period recorded for preadult ones kept at the same condition.

The cercarial emergence was known to be influenced by many factors such as the species, or strain of *Fasciola* and or host snails, the susceptibility of snails to the parasite and the size, nutritional condition, population density and breeding condition of infected snails, such as temperatures[22]. Accordingly, the results of the present study would not be simply compared with those reported on other species and or strains of *Fasciola* and snails kept at different conditions. Kendall and McCoullough[6] found that the cercarial emergence occurred throughout a wide range of temperatures above 10°C. So, in the present study the prepatent period of *F. gigantica* inside its snail host and the cercarial emergence might have delayed at temperature below 20°C.

Infection of snails with 2 miracidia gives more metacercariae in all groups in this study. On the relation between the number of inoculated miracidia and the produced cercariae, the result of previous authors are divergent: Itagaki and Itagaki[19] found that, the total number of cercariae passed by 10-miracidia infected group was almost the same as by 1-miracidium infected group because rediae were too many to be sufficiently nourished for development. The number of cercariae emergent from *L. peregra* infected with single and two *F. gigantica* miracidia was 1971 and 3395, respectively[4]. Lee *et al.* [20] stated that infection with 3 miracidia per snail produce more cercariae than infection 1 or 5 miracidia at different temperature and with different snail sizes. In contrary, Dreyfuss *et al.*[23] found that in *L. truncatula* infected by (1, 2, 5, 10, or 20 miracidia per snail) of *F. hepatica*, the highest metacercariae productivity for each miracidium was found in single-miracidium infections, Single-miracidium infections were the most effective, as the mean number of cercariae was the same as in other groups, whereas their survival rate was much higher.

Dreyfuss and Rondelaud[24] recorded 17.1 to 32.7% of the floating *F. gigantica* metacercariae produced from three different population of *L. trancatula*, this percentage was higher than recorded for *F. hepatica* (the percentage did not exceed 10%). They suggested that the percentage of floating cysts was trematode dependent and that the species of the snail used for the experimental infection had also an influence on 26.7% in *L. natalensis*[25] and 29.3% in *L. tmentosa*[24]. In the present study it was found that increasing in temperature resulted in highly significant increasing in the percentage of the floating metacercariae and when taken into an account that only one species from the snails and trematode were used, this may be due to the need of cercariae for rapid encystment if the required surface is not yet available.

The temperature, number of miracidia inoculated to the snails or snail age had no significant effect on the percentage of the snails which shed or those which not shed cercariae. In this regard Boray[26], Dreyfuss and Rondlaud[21] referred the frequency of cercarial shedding snail to the particular population of snails and trematode species. However, Sindou *et al.*[27] found that the absence of shedding was due to the tissue lesions, which appeared in the snail after miracidial penetration and developed over some weeks until snail death, under these conditions the physiological state of the intermediate host would not permit cercarial shedding[21].

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