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PARASITE SNAIL-HOST RELATIONSHIP OF FASCIOLA GIGANTICA AND LYMNAEA NATALENSIS

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ABSTRACT

The snail-host parasite interaction between *L. natalensis* and *F. gigantica* was studied with particular emphasis on the effect of the relationship on the reproduction of the snail-host. Snails were exposed to 4 *F. gigantica*miracidium each and maintained on blanched and dried lettuce in aquaria at a temperature of 28-30°C. Growth, production of egg and shedding of metacercariae were monitored.

Growth rate was reduced by about 19% in the infected snails relative to the uninfected control. Eggs were produced by the infected and uninfected control snails. However, it was observed that some of the masses produced by the infected snails did not contain eggs. Metacercariae production was found to suppress egg production. A negative linear relationship was discovered to exist between growth rate and metacercariae production with the latter being responsible for a 7% reduction in the growth of snails.

These findings confirmed the fact that the parasite has a negative effect on the snail-host. However contrary to earlier observations, infected snails produced eggs although at a reduced rate. The present findings have provideda new insight into the dynamics of F. gigantic snail-host interactions.

INTRODUCTION

One of the most exciting and productive areas of research during the past decade has been the investigations into the nature of snail-host susceptibility to their parasite. Experimental studies have demonstrated among other findings that extensive interaction occurs between snail plasma and the surface of the parasite (1, 2). The basic and fundamental studies on snail hosts represent an advancement of our understanding of the snail parasite relationship (3, 4). The interactions and the relationship of the snail-hosts of *Schistosoma*on infection of the parasite is a well documented phenomenon. The literature in this area of study has been reviewed (5); similarly, the interaction between F. hepatica has received much attention (6-11).

In spite of these there is paucity of information on the snail-host/parasite interaction of *Lymnaeanatalensis* and *Fasciolagigantica* or are scanty (12-14).

Routine maintenance of *Lymnaeanatalensis* population and their interactions with the parasite F. gigantic is important for research on fascioliasis.

This study examines the snail parasite interaction of **L. natalensis** and **F. gigantica** with emphasis on the effect of the infection on reproduction in the snail-host.

MATERIALS AND METHODS

Snail

The **L. natalensis** snails were collected from the Unversity of Ibadan botanical garden ponds in October, 1991. Identification of the snail species was based on earlier description (15).

Snail Culture

The snails were kept in glass aquaria (1.2 x 0.8 x 0.4m) and were placed on a wooden platform 1.0m above ground level. The aquaria were filled with dechlorinated tap water (DTW). Artifical aeration by means of pump was not done. The snails were fed on blanched and dried lettuce (15).

Production of Experimental Snails

Egg masses, the jelly-like substance in which the eggs are laid were collected by placing 8 adult snails in plastic bag containing 500 ml of DTW and allowing them to oviposit for 7 days. The egg masses were isolated by cutting the plastic bag around each egg mass. Egg masses wre kept in a glass beaker with one litre of DTW. They hatched after 2 weeks and reached infection size (10mm) in 10 weeks.

Snail Infection with *Fasciola gigantica* miracidia

Fasciola gigantica eggs were obtained from the bile of infected cattle which were slaughtered at the local abattoir. The bile was washed through a 250 μm aperture sieve. The eggs were collected into a beaker with tap water. The egg suspension was aliquoted 5-10 ml into test-tubes and incubated by a known method described by Frandsen 1975 at 28°C. **Fasciola gigantica** miracidia were hatched after 21 days as earlier described (10).

L. natalensis snails with shell-height 10mm were used. Each snail was exposed to 4 miracidia. After 2 hours of exposure all infected snails were returned to the aquaria.

Experiment Design

Young snails of smiliar sizes were randomly divided into 4 groups (A-D) of 20 snails each. Snails from three of the groups (A, B and C) had been exposed to 4 **F. gigantica** miracidia each. Group D was used as an uninfected control. About 6 weeks after infection of snails, cercariae were shed naturally in the 3 groups of the infected snails. Immediately shedding of cercariae was noticed by their circular dashing movements the snails were transfered into clean 2 litre aquaria. These were line with transparent adhesive tape at the air-water interface (16). The shed cercariae attached to the tape for encystation, so that when the tape was peeled off, the metacercariae were counted weekly

under a disecting microscope. Shell height was measured to the nearest 0.1 mm at the beginning of the experiment and every two weeks for 24 weeks. Egg masses and the number of eggs laid were also recorded weekly for 15 weeks infected and uninfexted groups of snails.

RESULTS

This method used for maintaining the snails was successful for breeding large number of snails. They increased at a rapid rate such that by 120 days of breeding the number had increased from the initial population of 15 snails to about 564 snails.

Survival of Infected Snails

All the snails exposed to miracidia became infected. Death rates among infected snails was high compared to the uninfected control. While 30, 35 and 30 percent mortality rates were recorded for the infect groups A, B and C respectively at the end of the experiment, 20 percent was recorded for the control group (Table 1).

Table 1: Survival of Snails in infected and uninfected groups

Weeks post exposure	Group A n = 20	Group B n = 20	Group C n = 20	Control n = 20
1	20	20	20	20
2	20	20	20	20
3	20	20	20	20
4	20	20	20	20
5	20	18	16	20
6	20	18	16	18
7	17	18	16	18
8	15	16	16	18
9	14	16	16	18
10	14	16	16	18
11	14	16	16	17
12	14	15	15	17
13	14	15	15	17
14	14	15	15	16
15	14	13	14	16
Death rate	30%	35%	30%	20%

The difference in the mean death rate between the infected and uninfected control was significant ($P < 0.05$).

Growth of the Snails

Fig 1 shows the growth of the four groups of snails measured by the shell height. There was no difference in all the groups until week 4 when the growth was reduced in the infected groups by 18.6%, 20.9% and 18.6% in groups A, B and C respectively relative to the non-infected group

the mean egg masses produced by groups A, B and C throughout the 15 weeks of this experiment were 39.6 ± 1.7, 41.0 ± 4.2 and 35.3 ± 2.7 respectively, a means of 1586 ± 1.9 egg masses was recorded in the non-infected group (Table 2).

The mean number of eggs laid by the uninfected group was 554.7 ± 4.8 which

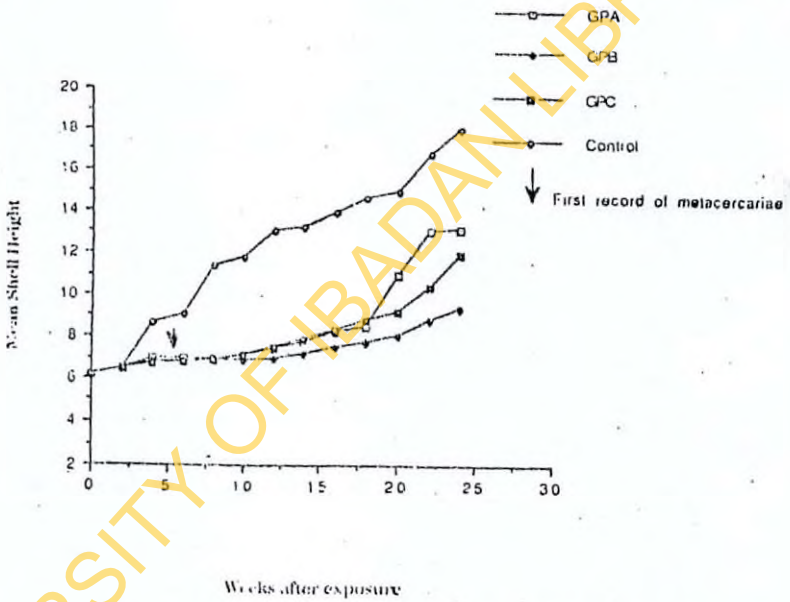

Fig. 1: Mean growth rate (x + SD)mm of groups of snails infected with F. gigantica miracidia.

Production of Eggs and Egg Masses

Eggs were not produced by the infected groups until seven weeks post infection, whereas production of eggs was first recorded at 3 weeks in the control group. There was a significant difference ($P < 0.05$) in the number of egg masses and number of eggs laid between the infected and the non-infected group. While was significantly ($P < 0.5$) higher than the mean number of eggs laid by the infected group of 135.1 ± 0.6, 182.9 ± 4.0, 170.1 ± 2.8 in group A, B and C respectively (Table 3). The mean number of egg masses laid per adult snail per day were 0.6 ± 0.3, 0.5 ± 0.3 and 0.4 ± 0.2 in groups A, B and C respectively; while that of the uninfected control group was 1.4 ± 0.4. The mean number of eggs

Table 2: Total egg masses produced by the groups of infected and uninfected snails.

Weeks post exposure	Group A	Group B	Group C	Control
1	0	0	0	0
2	0	0	0	0
3	0	0	0	84
4	0	0	0	126
5	0	0	0	168
6	0	0	0	210
7	12	13	13	139
8	52	45	56	151
9	78	78	67	176
10	29	34	29	178
11	39	11	22	202
12	78	74	21	214
13	78	84	74	238
14	88	95	74	202
15	98	100	78	202
Total	555	533	494	2,538
Mean ± SD	37.6 ± 1.7	41.0 ± 4.2	35.3 ± 2.7	158.6 ± 2.7

The difference in the mean values between the infected and uninfected control were significant ($P < 0.05$)

produced per adult snail per day for groups A, B and C were 2.6 ± 1.5, 2.6 ± 1.4 and 2.3 ± 1.4 respectively. The number of eggs for the uninfected control was 5.5 ± 0.8.

Production of *F. gigantica* metacercariae

Metacercariae shedding was noticed on day 37 after infection in all the groups of snails. Table 4 shows the production of metacecariae from the three groups A, B and C. There was a steady increase in the production from week 6 (37th day) to week 8 after which there was a significant ($P < 0.05$) drop in all the groups by weeks 9 and 10. Thereafter there was a rise in production again between weeks 11 and 13 and then there was a sustained drop till the end of the experiment. The peak metacerial production was recorded in week 8 in all the groups with a mean of 2,783. + 12.5, while the lowest production was recorded in week

Table 3: Total eggs produced by the groups of infected and uninfected snails.

Weeks post exposure	Group A	Group B	Group C	Control
1	0	0	0	0
2	0	0	0	0
3	0	0	0	426
4	0	0	0	768
5	0	0	0	852
6	0	0	0	680
7	140	140	138	686
8	445	440	446	692
9	421	433	480	656
10	80	72	84	636
11	40	38	48	642
12	336	356	345	640
13	378	379	372	620
14	272	270	253	640
15	210	242	216	620
Total	1,891	2,378	2,382	8,871
Mean ± SD	135 ± 11.6	182.9 ± 4.0	170.1 ± 2.8	554.7 ± 4.8

The difference in the mean values in the infected and uninfected control were significant ($P < 0.05$).

10 with mean value of 396 ± 38.6. The mean metacercariae produced per adult snail per day was 56.8 ± 32.

Relationship between egg production and metacercarial shedding

Figs (2 and 3) show the relationship between eggs laid/egg masses produced and the metacercariae shed by the infected snails. There was a non-linear inverse relationship between the egg/egg masses and the metacercarial production especially during the weeks of high production. The mean number of eggs laid per adult snail per day was reduced between weeks 10 and 11 amounting to 0.75 ± 0.5 and 0.4 ± 0.3 respectively. The number of metacercariae produced per adult snail per day during this period was very high amounting to 103 and 85 respectively. Metacercarial production therefore seemed to have had a drastic reduction on egg/egg masses produced by the snails.

Table 4: Production of metacercariae from 3 groups of Lymnaea natalensis exposed to F. gigantica miracidia

Week post exposure	Group A	Group B	Group C
6 (37th day)	292	379	413
7	2,860	1,835	1,842
8	2,872	2,838	2,641
9	400	786	381
10	440	368	380
11	2,552	3,442	2,680
12	2,316	2,412	2,456
13	2,400	2,072	2,311
14	1,108	1,634	1,860
15	736	993	868
16	560	732	684
Total	16,536	17,491	16,516

The relationship between the growth rate of the snails and metcaercariae shedding

Fig 4 shows the regression analysis of the relationship between the growth rate of the experimentally infected L. natalensis and the number of metacercariae shed. The slope of the regression shows a negative linear relationship between the growth rate and the metacercariae shed. $Y = 7.2065 - 8.2870\ e^{-5x}$ This indicates that the production of metacercariae by the snails had retardation (negative) effect on the growth of the snails. However, the metacercarial shedding had only a 7% reduction effect on the growth of the snails $R^2 = 0.070$.

Relationship between eggs laid and egg masses produced

Fig 5 shows this relationship between eggs laid and egg masses produced. There was a direct and non-linear relationship between eggs laid and egg masses produced. This was sustained till week 12 after when there was an inverse relationship. It was obseved that while the egg masses produced was increasing, the actual number of eggs laid was decreasing indicating that although the production of egg masses per adult snails per day increased gradually after week 12 of exposure of snails to F. gigantica miracidia, the actual number of eggs laid decreased gradually within this period.

DISCUSSION

This study shows that Lymnaea natalensis is highly prolific and can survive with or without artifical aeration. It is also worth nothing that unlike earlier practices (4, 14, 17) they did not need any special feeding on lettuce, tetramin fish food and/or a modified calcium alginate. In this study, the snails received no speical feeding apart from blanched and dried lettuce.

Growth was reduced in the infected groups compared to the non-infected control group as also observed before (14) and although this trend was more marked in this study.

Whereas growth was comparable in all groups until week 6 in one study (14), it was only comparable until week 4 in all the groups in this study. These observations may have been due to the difference in quantity and quality of feed. Growth in snails is a reflection of the quantity and quality of food available to the snail (4).

Egg production was recorded in the infected and the non-infected control groups of snails although the onset of production was delayed in the infected snails compared to the uninfected control. This observation is in contrast to the findings of other workers who did not observe any egg production in infected group of snails in the course of their experiments (12, 14). These workers observed complete stoppage of egg production among infected snails in their work; but here only a delay in onset and

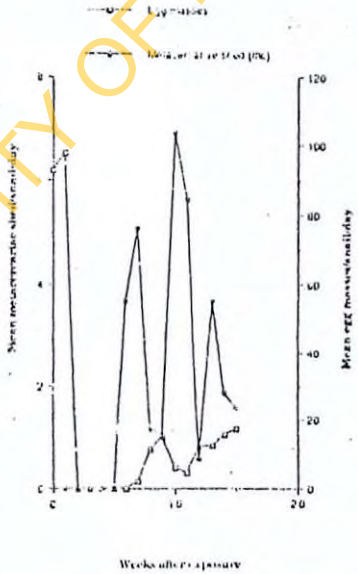

Fig. 2: Relationship between egg masses and metacercariae shed/adult snail/day.

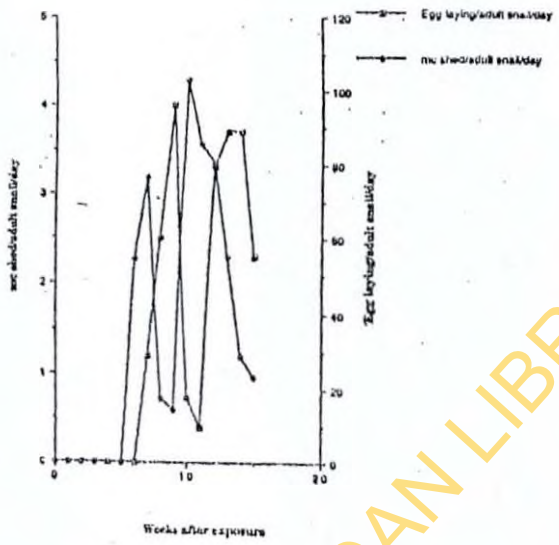

Fig. 3: Relationship between egg laying and metacercariae shed/adult snail/day.

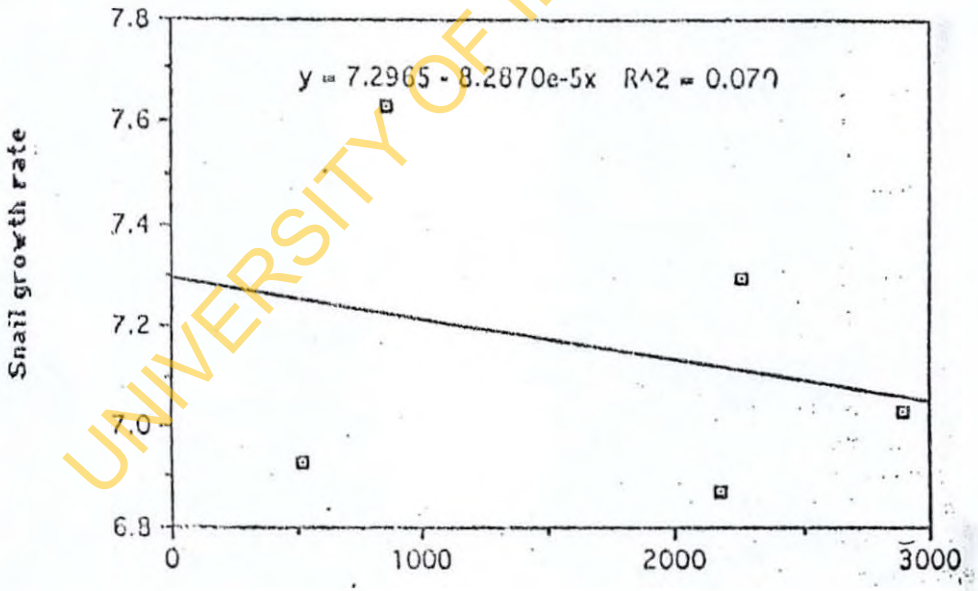

Fig. 4: Regression of snail growth rate against metacercariae production.

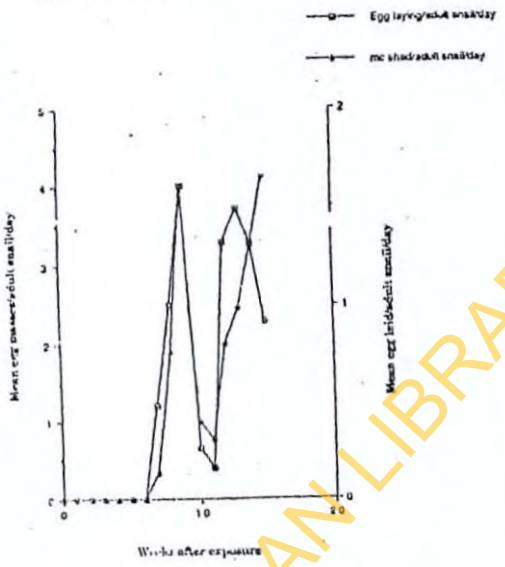

Fig. 5. Relationship between eggs laid and egg masses produced.

reduction in egg production was observed.

There was also an early onset in the production of metacercariae in this study as earlier reported (17) but contrary to the recommended 70 - 72 days for the production of cercariae by infected L. natalensis (18).

The inverse relationship between egg laying and metacercariae shed was not unexpected as complete cesastion of egg production in truncatula/Fasciola hepatica hard been observed (19). However, the production of metacercariae by the infected snails led to a drastic reduction in the eggs laid in this study. The study also showed that the effect of the metacercarial production is more related to the absolute number of eggs laid rather than the production of egg masses. This is because as observed in the experiment, although the egg masses produced increased steadily after a period of time, the actual number of eggs within these egg masses were very few. This is typical of laboratory infection that the net rate of egg laying is not a linear function of the egg masses produced (11).

The regression analysis of the relationship between growth and metacercariae shed showed that there is a negative linear relationship with a 7% reduction effect on growth. This also agress with the previous observation (14) that there is a reduction in the growth of the infected snails compared to the non-infected control particularly during periods of metacercarial production.

The differences observed in this study and those of other workers especially the production of eggs by infected snails may be due to several factors. In the first instance, this study was done in a tropical environment, in conditions that were very similar to what obtains in the natural tropical situtaions under which such snail-parasite interaction normally occurs. Besides, there is the possibility of a yet-to-be identified strain difference in the parasite and/or the snails. However, the present findings may introduce new variables into the dynamics of F. gigantica transmission in nature.

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