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SOME BIOLOGICAL CHARACTERISTICS OF *LYMNAEA TRUNCATULA* AND LABORATORY PRODUCTION OF *FASCIOLA HEPATICA* METACERCARIAE

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ABSTRACT: A study was made on some aspects of the biology of *Lymnaea truncatula* and production of metacercariae of *Fasciola hepatica* under laboratory conditions. The experiment was conducted using laboratory bred snails originally collected from Debre Brihan area. The snails were maintained on blue green algae (*Nostoc vascorum*) grown in the laboratory. Snails laid masses of eggs with mean±SD number of 7.8±2.8 eggs per capsule, 96.7% of them having one egg cell per egg. Hatchability was 86.9%. Mean age at maturity was 61.8±11 days. Survival of snails post-infection was inversely related to the miracidial infective dose. Higher proportion of snails infected with small number of miracidia shed more cercariae than those infected with larger number of miracidia. Young snails were less susceptible to infection and survived longer than adult snails post-infection. Younger snails also shed lower number of cercariae than the fully-grown adult snails. The study showed that both young and adult snails can be infected with miracidia and shed cercariae at different levels. Both the age of snails and miracidial infective dose influenced the susceptibility of snails to miracidial infection and production of cercariae.

Key words/phrases: Cercaria, *Fasciola hepatica*, *Lymnaea truncatula*, miracidium

INTRODUCTION

Snails belonging to the genus *Lymnaea* are considered to be exclusive intermediate hosts of fasciolosis, an economically important helminth parasite of grazing livestock throughout the world. Fasciolosis causes widespread mortality and morbidity in cattle, sheep and goats. *Lymnaea truncatula* transmits *Fasciola hepatica* in temperate areas and high altitude regions of tropics and subtropics while *Lymnaea natalensis* transmits *Fasciola gigantica* mainly in lowland areas (Over, 1982; Tibor, 1999).

In Ethiopia, *F. hepatica* is causing serious damage to livestock production particularly in highland areas where *L. truncatula* is abundant. *L. truncatula* flourishes during rainy season of the year spreading to marshy areas within a few weeks (Goll and Scott, 1979). Surveys done by several workers indicated high prevalence of fasciolosis in the country both in cattle and sheep (Bahru Gemechu and Ephriem Mammo, 1979; Graber, 1975; Getachew Tilahun, 1987; Yilma Jobre, 1985; Mezgebu Merid, 1995; Wassie Molla, 1995). The recent increase in small-scale irrigation schemes throughout the country is also anticipated to increase the risk of fasciolosis and other snail-borne diseases of animals and humans through creating favorable environment for snail intermediate hosts. Michael Asrat *et al.* (2005) reported that prevalence of ovine fasciolosis was significantly higher in the irrigated sites compared to non-irrigated sites.

Some workers have reported the economic impact of fasciolosis in Ethiopia. An annual loss of 350 million Birr was estimated by fasciolosis through decreased productivity alone in cattle (Bahru Gemechu and Ephriem Mammo, 1979). Ngategize *et al.* (1993) also estimated the annual loss due to ovine fasciolosis in Ethiopian highlands to be 48.4 million Birr. These losses are caused through mortality, liver condemnation at slaughterhouses, poor weight gain, infertility, reduction in traction power of oxen, and low weight at birth (Bahru Gemechu and Ephriem Mammo, 1979; Ngategize *et al.*, 1993).

Treatment of infected animals with flukicidal drugs and the use of molluscicides to kill snail intermediate hosts are among the methods of control for fasciolosis (Soulsby, 1986). Despite the huge economic losses incurred and the widespread distribution of fasciolosis in the country, routine treatment of clinically sick animals is the only method so far being practiced. Control measures

targeted to both the parasite and intermediate host to prevent infection is better than routine treatment of clinical illnesses of infected animals. Thus, information about biology and ecology of snail intermediate host is essential in designing control strategies of the disease. However, little work has been done on the dynamics and biological properties of the intermediate host of this parasite in Ethiopia. This paper presents a work on laboratory breeding of *L. truncatula* and its experimental infection with miracidia of *F. hepatica.*

MATERIALS AND METHODS

Snail collection and laboratory maintenance

This work was done in the Animal Health and Zoonotic Diseases Laboratory of the Aklilu Lemma Institute of Pathobiology, Addis Ababa University, from July to December 2003.

L. truncatula snails used for the experiment were originally collected from Debre Birhan area, 130 km North of Addis Ababa located at an altitude of 2780 meters above sea level. The mean annual temperature of Debre Brihan is 12.6°C where the minimum and maximum temperatures are 6.3°C and 18.8°C respectively. The average annual rainfall is 956 mm and the relative humidity is 59.6%.

The snails were maintained on blue green algae (*Nostoc vascorum*) grown in petridishes or plastic lunch boxes. They were regularly supplied with fresh algae. Algae were grown in the laboratory on mud prepared from the soil collected from the area of origin of snails. The mean minimum and maximum relative humidity of the laboratory during the study period were 49.3% and 52.3%, while the mean minimum and maximum temperature were 11.3°C and 23.1°C, respectively.

Eggs and hatching status

Egg capsules were picked every morning and kept in separate petridishes covered with moist filter paper. For each egg capsule, the date at which it was laid was labeled on the petridish. The number of eggs per capsule and the number of egg cells per eggshell was observed under dissecting microscope and recorded. Eggs were checked daily for hatching starting from day 10 after oviposition. This date was selected based on repeated preliminary observation made on commencement of egg hatching in the laboratory before the actual experiment was started. No egg was observed to hatch before 10 days.

Age at maturity

Young snails with known date of hatching were kept in wet petridishes and observations were made daily starting from day 30 post-hatching for the commencement of egg production. The date when a single egg capsule was detected in a container was taken as maturity date for that group of snails.

Infection with miracidia

Samples of liver and gall bladder of sheep infected with *Fasciola hepatica* were collected from Addis Ababa Abattoir. Eggs were harvested from the bile of infected sheep. The harvested eggs were then kept at room temperature in water from which miracidia hatched within 15-20 days. Snails were exposed, *en masse,* to different numbers of freshly hatched miracidia in petridishes filled with water for an hour. Batches of uninfected control snails were kept under similar conditions. For the experiment on the effect of miracidial infective dose on survival of snails and cercarial shedding, 30-40 days old snails were randomly allocated to different groups.

Cercarial shedding and survival of snails

Infected snails were checked for cercarial shedding by exposing them to light for 2 hours once every 10-15 days as of day 25 post-exposure in small glass vials filled with water. The number of surviving snails was recorded every 10-15 days post-exposure for both infected and control snails.

Data analysis

Chi-square was used to compare the rate of survival of snails at different days post-exposure, to check for the association between exposure of snails to various doses of miracidia and the rate of survival. It was also used to test if age of snails at the time of exposure to miracidia has significant effect on the rate of survival of snails and cercarial shedding. Poisson regression with number of deaths as dependent variable was used to estimate the risk of mortality at various days following exposure after adjusting for the baseline age.

RESULTS

For the experiment conducted to generate data on composition and hatching rate of snail eggs, 65 egg capsules containing a total of 507 eggs were harvested. The number of eggs per egg capsule varied between 4 and 14 and the mean±SD number of eggs per capsule was 7.8 ±2.8. Eight eggs (1.6%) had no egg cells, 5(1%) have 2 egg cells and 4 eggs (0.8%) were observed with 6–12 egg cells per eggshell. The remaining 490 eggs (96.7%) had a single egg cell per eggshell. Eggs started hatching 11 days post-oviposition and continued up to 26 days. Most eggs hatched between 14-19 days and, the pick hatching period was around 16 days (Fig.1). The overall hatchability was 86.9%. There was considerable variation in the length of time required for eggs to hatch even among eggs in a single egg capsule. When observed under the microscope, eggs in the same capsule were not at the same developmental stages even on the first day of oviposition.

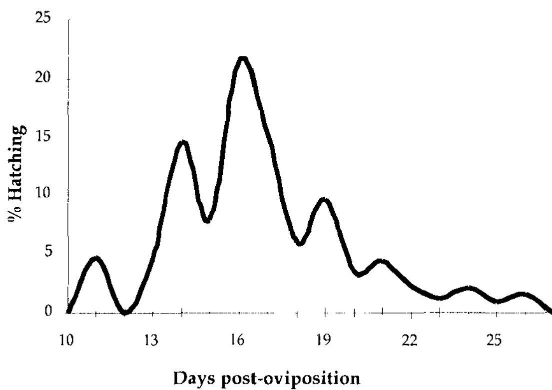

Fig. 1. Hatching rate of eggs of *L. truncatula* at room temperature.

Snails started laying eggs on day 46 post-hatching in some groups and the longest period observed was 76 days. The mean age at maturity was 62.1±11 days. With regard to infection with miracidia, snails infected with 20 miracidia each survived significantly less than those infected with 15 miracidia and those infected with 15 miracidia survived less than the snails infected with 10 miracidia. The higher the number of miracidia used to infect the snails, the shorter was the survival of snails (Table 1) and the lower was the total amount of cercariae shed (Table 2). Of the few remaining snails exposed to large number of miracidia, very low proportion or none were seen shedding cercariae.

Ten out of 10 (100%), 2 out of 10 (20%), and none out of 10 (0%) were shedding cercariae on 40th day in snails randomly picked from those exposed to 10, 15 and 20 miracidia per snail, respectively. On day 55 four out of ten (40%), two out of ten (20%) of the snails exposed to 10 and 15 miracidia per snail respectively were found shedding cercariae while no snails were seen shedding cercariae in groups exposed to 20 miracidia per snail (Table 2).

Table 1. Effect of miracidial infective dose on survival of snails post-exposure.

Number of miracidia per snail	Number (%) of snails survived after			Chi-square	*P-value*
	40 days	*60 days*	*75 days*		
0 (n=50)	40(80)	34(68)	30(60)	4.77	0.092
10 (n=50)	27(54)	12(24)	9(18)	17.1	0.000
15 (n=50)	17(34)	10(20)	7(14)	6.01	0.050
20 (n=50)	13(26)	8(16)	6(12)	3.52	0.172
Chi-square	25.83	40.44	40.54		
P-value	0.000	0.000	0.000		

Table 2. Effect of *F. hepatica* miracidial infective dose on cercarial shedding of snails on 40th and 55th days post-exposure.

Number of snails	Number of miracidia per snail	% of snails shedding cercariae on days 40 and 55 post-exposure	
		40 days	*55 days*
50	0(Control)	0	0
50	10	100	40
50	15	20	20
50	20	0	0

Age of the snails has shown statistically significant effect on survival of infected snails beyond 30 days of infection (P<0.05). Young snails seem less susceptible to infection than fully-grown adult snails. From Poisson regression, after adjusting for the duration post-exposure, the risk of mortality of snails exposed at age of 30, 40 and >60 days were 1.57, 2.73 and 3.09 times that of snails exposed at age of 15 days respectively. On the other hand, adjusting the age at exposure, the risk of mortality at 30, 40 and >60 days post infection was only 1.12, 1.24 and 1.62 times the risk of mortality at 15 days post-infection, respectively. All adult snails older than 60 days at the time of infection lived for short period once they started shedding cercariae, and all of them died before 45 days post-infection (Table 3).

Table 3. Effect of snail age at the day of exposure on survival of snails exposed to 10 miracidia of *F. hepatica* per snail.

Age of snails (days)	Number (%) of snails survived on various days post-exposure			
	20 days	*30 days*	*45 days*	*60 days*
15 (n=40)	32(80)	28(70)	15(37.5)	11(27.5)
30 (n=40)	29(60.4)	27(56.3)	12(25)	9(18.75)
40 (n=40)	29(72.5)	22(55)	13(32.5)	4(10)
>60 (n=40)	26(65)	14(35)	0	0
Chi-square	4.45	10.05	18.54	13.75
P-value	0.217	0.018	0.000	0.003

The development of trematodes in the snail was also slow in younger, small sized snails than in fully-grown adult snails (Table 4). A few young snails infected at 15 days of infection took longer time to shed cercariae almost twice that required in adult snails. Moreover, the number of cercariae obtained from such young snails was low. Fully grown adult snails infected at ages older than 60 days started shedding cercariae at 30 days post-infection and most of them were seen shedding large number of cercariae within a few days. Mortality of these snails increased as of the day they started shedding cercariae and all of them were dead by 45th day post-infection.

Table 4. Effect of snail age at the day of infection on cercarial shedding of snails exposed to 10 miracidia of *F. hepatica* per snail.

Age of snails (Days)	% Of snails shedding cercariae post-exposure			
	30 days	*40 days*	*50 days*	*60 days*
15(n=40)	0	0	10	20
30(n=40)	0	28.6	43.5	*
40(n=40)	0	20	40	*
>60(n=40)	80	90	*	*

* Stands for absence of data due to death of most of the snails.

DISCUSSION

The mean number of eggs per capsule observed in this study agrees with the counts reported by Hyman, (1967). However, the maximum number in this study is much lower than in other *Lymnaea spp.* Cecilia and Kelly (2000) also reported significant variation of the mean number of eggs per egg capsule produced by *L. columella* maintained on different feed sources. The number of egg cells per eggshell was also similar to the counts reported by Boer and Lever (1967) and Mulugeta Abebe (1992) for *L. stagnalis* and *L. natalensis,* respectively, where most egg capsules contained one egg cell per eggshell. In the present study, 1.6% of the eggshells were with no egg cell. This finding is not in agreement with the observations made by the previous workers and could be a unique characteristic of *L. truncatula.*

The hatching rate of 86.9% observed in this study is higher than that observed for eggs of *L. natalensis* (Mulugeta Abebe, 1992). This could be due to improved snail cultivation conditions than the previous work, which was reported to be contaminated with micro- and macro-organisms. It could also be due to species variation in hatching potential of the snails and other factors like feed and temperature. Percentage of hatching was reported to be dependent on the type of feed used by parent snails and the temperature at which the snails were maintained (Abdul Aziz and Raut, 1996). While some eggs started hatching as early as day 11, others remained unhatched until day 26. No egg was seen hatching after day 26 and about 75% of the eggs hatched between 14 and 19 days post-oviposition. The reason for such protracted period for hatching might be due to difference in developmental stages of eggs when laid, which was observed under dissecting microscope even in a single egg capsule. Mulugeta Abebe (1992) observed commencement of hatching for *L. natalensis* after 15 days and continued up to 40 days post-oviposition.

The mean period of 2 months for maturity of snails seems too long. This could be due to unfavorable laboratory conditions like temperature, humidity, light as well as feed that are determinant factors for growth and fecundity of snails. (Vander Schalie and Davis, 1968). Abdul Aziz and Raut (1996) reported that *L. luteola* took range of 25 to 93 days after hatching to attain sexual maturity under different environmental conditions. Earlier works showed the presence of considerable variation in growth and fecundity of snails under different culture conditions (Vander Schalie and Davis, 1965; 1968).

The direct parasitic effect by the developmental stages of the parasites in large number is the most likely explanation for the reduced longevity of snails exposed to larger number of miracidia and lower frequency of snails shedding cercariae observed in the present study. This finding is in agreement with that of Dreyfuss *et al.* (1999) which showed that the number of miracidia had a significant influence on snail survival post-exposure and the frequency of infected *L. truncatula* that died without shedding cercariae. The lower proportion of cercariae shedding snails

exposed to large number of miracidia may be due to mortality of most infected snails at early infection and the prolonged period required for the completion of developmental stages in highly parasite infected stunted surviving snails. The previous work showed that young *Lymnaea* snails infected by miracidia of *Fasciola hepatica* had smaller shell height compared to uninfected control (Busson *et al.*, 1982).

The reduced susceptibility of young snails to infection with miracidia of *F. hepatica* compared to the fully-grown adult snails is in agreement with the report by Smith (1984) which revealed an increase in proportion of infected snails, the mean redial burden and the proportion of mature rediae with increase in shell length of *L. truncatula*. It is also similar with the report of Smith (1987) that showed the probability of *L. truncatula* to remain uninfected following exposure to a single miracidium of *F. hepatica* is inversely related to snail size. However, it was contrary to what was reported by Monica and Otavio (2001) about infection of *Biomphalaria spp.*, in which sexually immature snails were more susceptible to infection with *Schistosoma mansoni* than mature adult snails. This could be due to species variation.

Although it was not possible to generate data on the longevity of snails, 60% of uninfected control snails maintained in similar laboratory conditions were able to survive for about 110 days (Table 1). The findings from Poisson regression showed that the risk of mortality due to infection was significantly higher in adult snails than in younger snails. Therefore, the significant mortality of infected adult snails 30 days post-exposure could not be attributed to natural death of snails due to old age. Rather it could be due to the parasitic effects of *F. hepatica* on the tissues of snails and damage caused by the release of large number of cercariae. Despite early death of adult snails compared to young snails, they were seen shedding considerable number of cercariae within short period of time. This result agrees with that of Kendall and Ollerenshaw (1963), which confirmed that, the size not the number of *Lymnaea truncatula* that influences the amount of the developmental stages of *Fasciola hepatica* present in the snail. The high abundance of adult snails (large snails) in a given grazing area is an indication of the risk of contamination of the grass by the infective metacercariae, provided that there are *Fasciola* infected animals grazing in the area.

In conclusion, the study showed that both young and adult snails could be infected with miracidia and shed variable number of cercariae. Size and age of snails as well as the number of infective miracidia have been shown to influence the susceptibility of snails to infection and subsequent production of cercariae. The use of fully-grown adult snails and lower number of miracidia is recommended for better laboratory production of metacercariae of *F. hepatica*.

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REFERENCES

1. Abdul Aziz, M.D. and Raut S.K (1996). Thermal effect on the life-cycle parameters of the medically important freshwater snail species lymnaea (Radix) luteola (Lamarck). *Mem. Inst. Oswaldo Cruz.* 91:119–128.
2. Bahru Gemechu and Ephriem Mammo (1979). A preliminary survey of Bovine fascioliasis in Ethiopia. *Eth. J. Agri. Sci.* 1:5–12.
3. Boer, M. and Lever, J. (1967). The effects of Amphenone B on egg production of *Lymnaea staginalis*. *Malacologia* 5:431–444.
4. Busson, P., Busson, D., Rondelaud, D. and Pestre Alexandre, M. (1982). Experimental data on the infestation of the young of 5 species of *Lymnaea* by *Fasciola hepatica* L. *Ann. Parasitol. Hum. Comp.* 57:555–563.
5. Cecilia, P.S. and Kelly, G.M. (2000). Rearing of *Lymnaea columella* (Say, 1817), Intermediate Host of *Fasciola hepatica* (Linnaeus, 1758). *Mem. Inst. Oswaldo Cruz.* 95:739–741.
6. Dreyfuss, G., Vignoles, P., Rondelaud, D. and Vareille Morel, C. (1999). *Fasciola hepatica*: Characteristics of infection in *Lymnaea truncatula* in relation to the number of miracidia at exposure. *Exp. Parasitol.* 92:19–23.
7. Getachew Tilahun (1987). A survey of fascioliasis in cattle sheep and goats slaughtered at Addis Ababa Abattoir. IPB research report, Addis Ababa University, pp. 310–11.
8. Graber, M. (1975). Helminths and helminthiasis of domestic and wild animals in Ethiopia. *Bul. Ani. Hlth. Prod. Afr.* 23:57–86.
9. Goll, P.H. and Scott, J.M. (1979). Fascioliasis in the Ethiopian Central Highlands, I. Dynamics of intermediate snail host population, miscellaneous report [illegible]

Overseas Pest Research Development. London, pp. 1–20.

10. Hyman, L.H. (1967). *The Invertebrates*. I. Mollusca. Vol. 1. Mc Graw-Hill Books, 603 pp.

11. Kendall, S.B. and Ollerenshaw, C.B. (1963). The effect of nutrition on growth of *Fasciola hepatica* in its snail host. *Proc. Nut. Sci.* **22**:41–46.

12. Mezgebu Merid (1995). A survey on ovine fascioliasis and lungworm infestation in Addis Ababa and the surrounding high land areas. DVM Thesis, Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, Ethiopia.

13. Michael Asrât, Beyene Petros, Yilma Jobre, Don, P.; Yoseph Shiferaw, Girma Tadesse and Mulugeta Mamo (2005). Infection prevalence of ovine fasciolosis in small-scale irrigation schemes along the Upper Awash River Basin. *Ethiop. Vet. J.* **9**:19–27.

14. Monica, A.F. and Otavio, S. (2001). Infection by *Schistosoma mansoni* Sambon 1907 in the first four months of life of *Biomphalaria straminea* in Brazil. *Mem. Inst. Oswaldo. Cruz.* **96**:185–192.

15. Mulugeta Abebe (1992). Preliminary Observations on Some Biological characteristics of *Lymnaea natalensis* (Basomatophora: lymnaeidae) an intermediate host of *Fasciola gigantica. Helmintologia* **29**:185–191.

16. Ngategize, P.K., Teklye Bekele and Getachew Tilahun (1993). Financial losses caused by fascioliasis in Ethiopian Highlands. *Trop. Anim. Hlth. Prod.* **25**:155–161.

17. Over, H.J. (1982). Ecological basis of parasite control, Trematodes with special reference to fascioliasis. *Vet. Parasitol.* **11**:85–95.

18. Smith, G. (1984). The relationship between the size of *Lymnaea truncatula* naturally infected with *Fasciola hepatica* and the intensity and maturity of the redial infection. *J. Helminthol.* **58**:123–127.

19. Smith, G. (1987). The relationship between the density of *Fasciola hepatica* miracidia and the net rate of miracidial infections in *Lymnaea truncatula. Parasitol.* **95**:159–163.

20. Soulsby, E.J. (1986). *Helminths, Arthropods and Protozoa of Domesticated Animals*, 7th ed. Bailliere Tindall, London.

21. Tibor, K. (1999). *Veterinary Helminthology*. Reed Educational and Professional Publishing Ltd, London, pp. 3–10.

22. Vander Schalie, H. and Davis, G.M. (1965). Growth and stunting in Oncomelania; Gastropoda: Hydrobidae. *Malacologia* **3**:81–102.

23. Vander Schalie, H. and Davis, G.M. (1968). Culturing Oncomelania Snails (Prosobranchia: Hydrobidae) for study of oriental schistosomiasis. *Malacologia* **6**:321–367.

24. Wassie Molla (1995). Prevalence of bovine and ovine fascioliasis: a preliminary survey in Nekemte and its surrounding areas. DVM thesis, Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, Ethiopia.

25. Yilma Jobre (1985). Study on ovine fasciolosis and other helminth parasites at Holleta, DVM Thesis, Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, Ethiopia.