Further Observations on the Susceptibility of Different Species of *Lymnaea* Snails of Iran to Miracidia of *Fasciola hepatica* and *Fasciola gigantica*

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Summary

Fascioliasis caused by Fasciola hepatica *and* F. gigantica *is widely spread in Iran. Intermediate hosts of these parasites, lymnaea snails, have an important role in transmission of the disease to animals and human beings. The degree of susceptibility of limnaea of Iran to infection with* F. hepatica *and* F. gigantica *has been investigated and is reported in this paper.*

Lymnaea truncatula *and* L. stagnalis *act as intermediate hosts for* F. hepatica, *whereas* L. Peregra, L. auricularia (gedrosiana), L. Palustris *and* L. Stagnalis *act as intermdeidate hosts for* F. gigantica, *in most parts of Iran.*

Introduction

Fascioliasis, caused by *F. hepatica* and *F. gigantica*, widely occurs in domestic animals in Iran. The disease in livestock and individual human beings has been reported by many workers. In 1989 an epidemic of human fascioliasis occurred in Iran, in the Caspian Sea region, where approximately 7000 of people were afflicted(1).

Lymnaea species, as intermediate hosts of *Fasciolae*, have crucial role in

completion of the life cycle and the transmission of the parasite to animals and man.

The common species of *Lymnaea* snails found in Iran are: *L. truncatula, L. peregra, L. auricularia, L. palustris, L. stagnalis, L. auricularia (gedrosiana)*. Recently *L. auricularia (rufescens)* was reported to be present in south and south-east of Iran(2).

Some of *Lymnaea* snails, intermediate host for *Fasciola* spp in Iran, have been previously studied(3, 4, 5).

L. truncatula is omnipresent in the Iranian plateau and appears to be the most suitable intermediate host for *F. hepatica* in this country, as it is in Europe(6). In order to further study *Lymnaea* and determine the degree of susceptibility of *Lymnaea* spp, other than previously studied, to *F. hepatica* and *F. gigantica* miracidia, the experiments described in this paper were conducted.

Materials and methods

The aquatic snails, *L. peregra, L. auricularia (gedrosiana), L. palustris* were investigated. All *Lymnaea* used for the present experiments were reared in the snail chambers at Parasitology Department, Razi Institute. The origin of *L. peregra* and *L. auricularia* was Mardabad swamps, 60 km west of Tehran. They had been in culture at the laboratory since 1967. The origin of *L. palustris* was Babol and Babolsar rice fields in northern Iran. They had been cultured at our laboratory since 1978. The strain of *L. stagnalis* was from Uremia, a province in north-west of Iran.

The eggs were collected directly from the uterus of the worms *F. hepatica* and *F. gigantica*. *Fasciolae* were collected from bile ducts of sheep livers. These sheep, which were sacrificed, had been artificially infected with either *F. hepatica* or *F. gigantica* metacercariae.

The collected *F. hepatica* and *F. gigantica* eggs were cultured separately, in dark petri-dishes containing water, at 24°C for 10 to 15 days until development of miracidia, thenafter they were kept at 4°C in a refrigerator.

According to recommendations by Kendall et al.(7), and our previous experience, only juvenile snails were exposed to newly hatched miracidia, because snails are more susceptibile to infection at this particular age.

In order to determine the snails susceptibility to infection with miracidia of *Fasciolae* , the following experiments were conducted:

Experiment I: 120 *L. peregra*, 40-60 days old, were used. They were divided in three groups:

Group A: 40 snails were individually exposed to 1 newly hatched *F. gigantica* miracidium.

Group B: 40 snails were individually exposed to 2 newly hatched *F. giganticia* miracidia.

Gropu C: 40 snails were individually exposed to 2 newly hatched *F. hepatica* miracidia.

Experiment II: 124 *L. auricularia* (*gedrosiana*), were divided into three groups and were infected as follows:

Group A: 22 fifty-day old snails were exposed to 2 *F. gigantica* miracidia.

Group B: 80 two- to four-week old snails were exposed to 1 single *F. gigantica* miracidium.

Group C: 22 two- to four-week old snails were exposed to 2 *F. hepatica* miracidia.

Snails were, individually, exposed to miracidia for 4 h in a test tube containing small amount of pond water. Each group of snails was kept in a small, wide-mouth plastic container. They were daily checked for development of radiae and cercariae.

Experiment III: 865 *L. palustris* of different ages were exposed overnight to, approximately, 4 miracidia of either *F. gigantica* or *F. hepatica* miracidia as follows:

Group A: 250 adult snails were exposed to *F. gigantica* miracidia.

Group B: 255 juvenile, 1- to 3-week old, snails were also exposed to *F. gigantica* miracidia.

Group C: 180 adult snail were exposed to *F. hepatica* miracidia.

Group D: 180 juvenile, 1- to 3-week old, were exposed to newly hatched miracidia of *F. hepatica*.

Each group of the exposed snails was kept in a snail room, in separate containers, and checked for development of rediae and cercariae.

Experiment IV: 360 adults and 360 juvenile *L. stagnalis* were exposed, according to the pattern described in Experiment III, to *F. hepatica* and
F. gigantica miracidia:

Group A: 240 adult snails were exposed to *F. gigantica* miracidia.

Group B: 240 juvenile snails, 1- to 3-week old, were exposed to newly hatched *F. gigantica* miracidia.

Group C: 120 adult snails were exposed to newly hatched *F. hepatica* miracidia.

Group D: The remaining 120 juvinile snails were exposed to *F. hepatica* miracidia.

Infected snails were kept, in separate containers, in a snail room and were checked daily for development of rediae and cercariae.

Results

L. peregra and *L. auricularia* (*gedrosiana*), in adult or immature stages, were very suitable intermediate hosts for *F. gigantica* miracidia and produced cercariae and metacercariae. Rates of susceptibility to infection, prepatant periods, from the time of exposure to miracidia till the first shedding of cercariae, number of cercariae produced when 1 or 2 miracidia were used are shwon in Table 1, for *L. peregra*, and in Table 2 for *L. auricularia*. Adult and juvenile *L. peregra* and *L. auricularia* (*gedrosiana*) were completely refractory to *F. hepatica* miracidia infection.

Rates of susceptibility to infection and prepatant periods, when 1 or 2 miracidia of *F. gigantica* were used, are shown in Table 3 for *L. palustris*. *L. palustris*, at adult stage, were completely refractory to *F. gigantica* and *F. hepatica* miracidia. They were also absolutely refractory to multiple exposure to *F. hepatica* miracidia. However, at juvenile stage, they were ussceptible to *F. gigantica*.

The adults of *L. stagnalis* were completely refractory to both *F. hepatica* and *F. gigantica* miracidia. On the contrary, the immatures of this snail were suscetible to both *F. hepatica* and *F. gigantica*. The results of the expetiment on this snail are summarised in Table 4. These results indicate that *L. stagnalis* is not a good intermediate host for animal fascioliasis.

Table 1. *Fasciola hepatica* and *Fasciola gigantica* infection rates in *Lymnaea peregra* exposed, 4 h, to 1 or 2 miracidia.

Experimental subjects	F. gigantica		F. hepatica
	Group A.	Group B.	Group C.
Lymnaea peregra number	40	40	40
Snail age (days)	40-60	40-60	40-60
Exposed miracidia (number)	1	2	2
Snail infection rate	33.3%	86.6%	0%
Prepatent period for cercaria	70	57	0
Cercaria shedding period (days)	47	65	0
Mortality rate in prepatent period	32%	25%	0
Total cercaria excretion per snail	1971	3395	0
Mean life of infected Lymnaea (days)	117	124	0
Daily cercarial output per snail (Mean)	42	55	0

Table 2. Observations on *L. auricularia* exposed, individually, in a test tube to 1 or 2 *F. gigantica* or *F. hepatica* miracidia for a period of 4 h.

Experimental subjects	L. auricularia (gedrosiana)		
	Group A.	Group B.	Group C.
Snail number	22	80	22
Lymnaea snail age	50	15-30	15-30
Exposed miracidia number	2	1	2
Fasciola species	F.gig.	F.hep.	F.hep.
Snail infection rates	38%	80%	0%
Prepatent period for cercaria (days)	70	56	0
Mortality rate in prepatent period	27.2%	29%	5%
Total excretion cercaria per snail	1164	1158	0
Daily cercarial output per snail (number)	48	35	0
Mean life-span of infected Lymnaea (days)	94	114	0

Table 3. Observations on susceptibility of *L. palustris* exposed, for 4 h, to one or two miracidia of *F. hepatica* and *F. gigantica*.

Experimental subjects	L. palustris			
	Group A.	Group B.	Group C.	Group D.
Snailnumber	250	255	180	180
Snail age (weeks)	adult	1-3	adult	1-3
Fasciola species	F.gig.	F.gig.	F.hep.	F.hep.
Number of miracidia per snail	4	4	4	4
prepatent period (days)	0	65	0	0
Mortality rate in prepatent period	0	11%	0	0
Infection rate	0	79%	0	0
Daily mean cercaria shedding rate	0	17	0	0
Mean life in infected snail (days)	0	89	0	0

Table 4. Susceptibility of *L. stagnalis* after exposure, for 4 h, to 4 *F. hepatica* and *F. gigantica*.

Experimental subjects	L. stagnalis			
	Group A.	Group B.	Group C.	Group D.
Miracidia source	F. gigantica		F. hepatica	
Snailnumber	240	240	120	120
Snail age (weeks)	adult	1-3	adult	1-3
Number of miracidia (per snail)	4	4	4	4
prepatent period (days)	0	58	0	71
Prepatent mortality	2%	22%	3%	79%
Infection rate in snails	0	75.8%	0	16.3%
Mean cercarial shedding per snail	0	11	0	47
Cercarial excerting period (days)	0	31	0	24

Discussion

According to Massoud et al.(4) the infection rates of *L. peregra* and

L. auricularia to *F. gigantica* were 73% and 45%, respectively. In the present studies we almost found similar results. In addition, we also found that immature stages of the above mentioned snails were more susceptible to *F. gigantica* miracidia and showed 86.6% infection rate. Furthermore, the rates of mortality in infected snails were 32% to 25% in the prepatent period.

Arfaa et al.(3) reported that *L. auricularia* (*gedrosiana*) was susceptible to *F. hepatica* miracidia. However, our observations showed that adult and immature *L. peregra* and *L. auricularia* were completely refractory to infection with *F. hepatica* miracidia. Massoud et al.(4) reported that the source of *Fasciolae* eggs from which miracidia were obtained was from a naturally infected liver of a buffalo which, they suspected, must have carried a mixed infection with *F. hepatica* and *F. gigantica*. On the basis of the present findings, we confirm the views of the latter authors and believe that Arfaa et al.(3) must had been dealing with *F. gigantica*.

Massoud et al.(4) reported that *L. palustris* was completely refractory to both *F. hepatica* and *F. gigantica* miracidia. In our study, we found that 1- to 3-week old *L. palustris* were susceptible to *F. gigantica* miracidia and produced cercaria and metacercaria which infected the animals, but were refractory to *F. hepatica*. We suspect that Massoud et al.(4) exposed adult snails which are completely refractory to both *Fasciola* species.

Our studies on *L. stagnalis* in Iran indicate that this species, which is abundant in north west and western parts of Iran, at the immature stage is susceptible to both *Fasciolae*. On the contrary, adults of *L. stagnalis* are completely refractory to both *F. hepatica* and *F. gigantica* miracidia (Table 4).

Moreover, as it is shown in Table 4 the number of cercariae shed by *L. stagnalis* was limited because all infected snails died during the few cercarial shedding days. Therefore, it seems reasonable to believe that this species can not maintain the transmission of the infection in nature.

We conclude that *L. truncatula* and *L. stagnalis* act as the snail intermediate hosts for *F. hepatica* but *L. peregra*, *L. auricularia* (*gedrosiana*), *L. palustris* and *L. stagnalis* act as the intermediate host for

F. gigantica, in most parts of Iran.

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