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Original Article

Inhibition Effect of pH on the Hatchability of *Fasciola* Miracidia under Laboratory Conditions

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Abstract

Background: Fasciolosis, caused by the liver flukes of the genus *Fasciola*, is one of the most prevalent diseases of domestic livestock and human throughout the world, imposing considerable economic losses. The present study was aimed to assess the effects of different pH values on hatching rate of *Fasciola* miracidia.

Methods: The flukes were isolated from the infected livers of the slaughtered ruminants at the abattoir of Urmia City, Iran, crushed thoroughly and sieved for isolation of the *Fasciola* eggs. The eggs were washed up several times by PBS (0.01N, pH 7.2). They were incubated at different pH values of 7±0.1 (control) and 3-9.5 (treatments) at 28°C for 16 days.

Results: The maximum hatching rate was observed at pH 7 (14.93±0.65%), while no miracidia were hatched at pH 3 and/or pH 9-9.5. There were significant differences between the hatching rate of the treatments and that of the control group.

Conclusion: Water pH is proven to be a crucial factor affecting the life cycle of *Fasciola* and its epidemiology.

Introduction

Parasitic infection is an important issue in veterinary and public health throughout the world, mainly in the developing countries (1). Fasciolosis is a cosmopolitan disease caused by the digenean trematodes of the genus *Fasciola*. Fasciolosis outbreak in humans and animals accompanies with reinfection, while no appropriative vaccine has yet been produced against the disease (2). *Fasciola* is an animal-borne disease whose epidemiologic feature has been completely changed in the recent years (3, 4).

The life cycle of the fasciolid trematodes starts with the eggs produced in the body of

definitive host and passes out of the liver and into the intestine, follows by the penetration of the miracidia into an intermediate host, mostly the freshwater snails like lymnaeid snails. From the snail, cercariae emerge and encyst as metacercariae on nearby vegetation. The metacercariae are ingested by the ruminant or, in some cases, by humans eating uncooked foods such as watercress or salad. The immature juvenile begins the process of excystment and burrows through the intestinal into the peritoneal cavity. The parasite browses on liver and finds its way to the bile duct, where it matures into an adult and begins to produce eggs (5). Environmental factors such as pH are critical for *Fasciola* life cycle including their embryonic development. The effect of pH on hatchability of trematode larvae in the aquatic habitats has a significant role (6). The laboratory assessment of the impact of pH on the production of *Fasciola* miracidia can be beneficial for further understanding of the degree of its effect in natural environments and distribution of the disease.

This study was aimed to evaluate the effects of different pH levels on egg hatchability in an Iranian isolate of *Fasciola*.

Materials and Methods

Fasciola sampling and egg isolation

Adult *Fasciola* were isolated from the infected livers of the slaughtered domestic ruminants in the abattoir of Urmia City in 2013, and transferred to the Laboratory of Helminthology of Faculty of Veterinary Medicine, Urmia University, Iran. The livers were cut into small pieces and adult *Fasciola* helminths were removed from the bile ducts. The isolated *Fasciola* were then examined preliminarily for the presence of the eggs by microscopic inspection, crashed in a mortar containing 10 mL of distilled water and sieved to gather the eggs. The eggs were washed several times using 0.086% Ringer's solution and centrifuged at 2000 rpm for five minutes. The supernatant was discarded and the precipitated eggs were washed three times (7). An amount of 1 mL of the residue containing approximately 1000 mature *Fasciola* eggs was considered as a single dose for the treatment groups. The required alkaline and acidic solutions were prepared, respectively from sodium hydroxide (0.1 N) and hydrochloride acid (0.1 N) by using a pH meter (Az8652, Taiwan).

Experimental design

The treatments included a single dose of the eggs in 20 mL of mineral water (pH 7±0.1) as control and in the water of different pH ranges (3-9.5) with three replicates for each treatment. The control group was also provided with a single dose of the eggs in mineral water (pH 7±0.1). The eggs of each treatment were incubated at 26±2 °C for 16 d. The pH of each treatment and the control group kept constant during the course of the study. On the last day, all the treatments were exposed to the light with a density of 100 Watts for 4-6 h to stimulate miracidia release (8, 9). The eggs were examined microscopically at 400× magnification to estimate their hatching rates.

Statistical analysis

One-way ANOVA and linear regression tests were used for evaluating the findings using the SPSS statistical program (version 14, SPSS Inc., Chicago, IL, USA). A *P*-value of < 0.05 was regarded as significant for all the comparisons.

Results

The process of the miracidia formation in the control group was prolonged for 16 d (Fig. 1). On the first day of the study, it was clear and a tangible cellular growth. The cellular mass division was not observed, but the cells began to amass from the second day. Developed embryonic cells were observed as large spots alongside of egg on the 6th day. They became uniformed and seen as large brown

spots on the 9th day. On the 12th day, the spots amassed in a corner of the egg. The embryonic tissue differentiation was completed and the miracidia stage of *Fasciola* was observed inside the egg. The miracidia were emerged through light stress for 20 min.

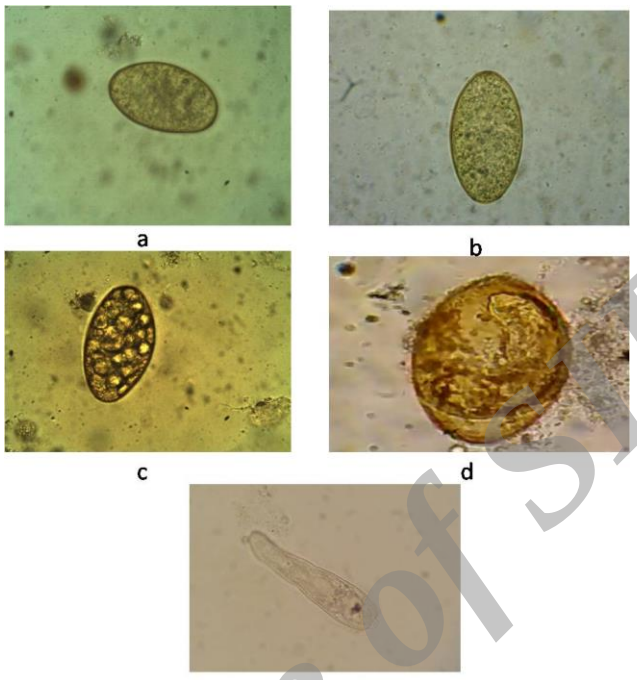

Fig.1: The growing stages of miracidia in *Fasciola* eggs (original): first day (a); sixth day (b); ninth day (c); twelfth day (d); sixteenth day (e) (magnification: 400×)

Maximum and minimum hatching rate were respectively observed at pH 7 (14.93±0.65) and pH 3 (0%) (Table 1). There was significant difference between average number of hatched and un-hatched eggs of *Fasciola* in both control and treatment groups ($P \leq 0.05$) (Table I). Hatching rates of all the treatments had significant correlations with pH values ($r = 0.865$) (Fig.2).

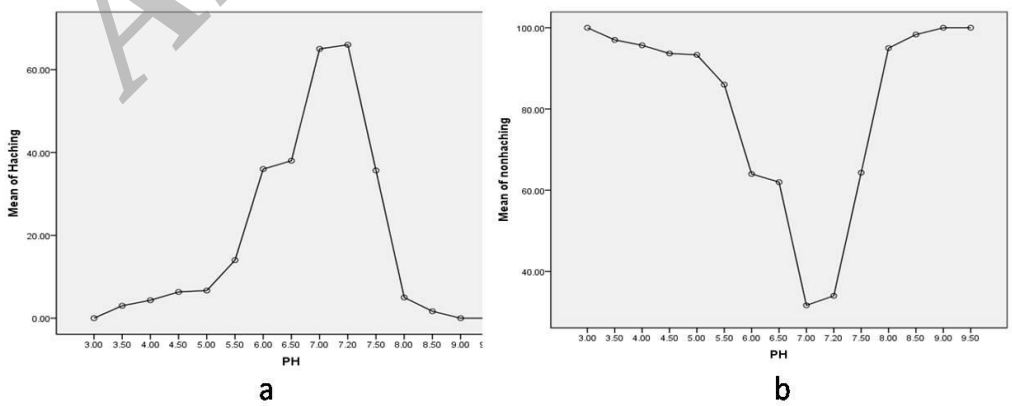

Fig.2: The average of hatched (a) and non-hatched (b) rates of *Fasciola* eggs at different pH levels.

Discussion

Environmental factors are important for different stages in the life cycle of parasitic flukes and as a result, their epidemiology. The water temperature adjusted for incubation of *Fasciola* eggs in the present study was proven suitable as judged by the earlier studies (10-12). However, appropriate temperature range for embryonic development is different for different *Fasciola* species (11, 13, 14).

Similar to our results, several previous studies have observed the maximum hatching rate of *Fasciola* miracidia at a neutral pH level (10-12). The hatchability of *F. gigantica* eggs decreased to 4-14% at pH of 5-10 (11). Several freshwater habitats in West Azarbaijan, northwestern Iran have a water pH range of 5.5-9 (15) which seem to be favorable for completion of the life cycle of parasitic trematodes. In the present study, the *Fasciola* eggs did not hatch at acidotic (pH 3) or alkaline (pH > 8.5) conditions. Rowcliffe and Ollerenshaw (11) and Al-Jibouri (12) reported null hatching percent of the miracidia at the pH values of less than 4.2 and over 9. Variations in pH levels affected the growth of the miracidia in *Fasciola* eggs. Several studies have shown the effects of higher and lower pH levels on the delay in hatching time, and increase in the number of infertile eggs of trematodes (10-12). Water pH can also have indirect impact on the life cycle and propagation of parasitic trematodes through its effect on the toxicity of heavy metals (16, 17).

From an epidemiological prospective, pH not only affects the life cycle of trematodes, but also impacts their intermediate hosts including snails. Distribution of freshwater snails depends on water qualitative conditions including pH (18-20). The pH range of 7.2-7.5 is well-suited for snail activity and propagation of snails' population in Shadegan Wetland, Iran (21). Low pH value might be fatal to snails living in freshwater (18). The alkaline pH of about 8 is suitable for multiplication of snails living in freshwater (19, 20).

Detailed studies on environmental preferences and effects of different ambient conditions on fasciolid trematodes and their distribution is crucial mainly because new *Fasciola* outbreaks have been reported from many countries (22), while, yet, there is no appropriate vaccine to induce immunity in the re-infected ruminants.

Conclusion

By understanding the ecological drivers of the growth and propagation of parasitic trematodes, it will be possible to take steps toward prevention and controlling the destructive effects of their outbreak. It can consequently overt damage to human and livestock and economic losses in the future.

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References

1. Rim HJ, Farag HF, Sornmani S, Cross JH. Food-borne trematodes: ignored or emerging? Parasitol Today. 1994; 10: 207-9.
2. Yakhchali M, Ghobadi K. A survey on helminths infection of liver and economic lost in slaughtered sheep of Urmia slaughterhouse, Iran. Iran J Vet Med. 2005; 11: 60-5 (Persian)
3. Muller R. Worms and Human Disease. Oxon, USA: CAB International Publishing; 2002.
4. Ashrafi K, Valero MA, Panova M, Periago MV, Massoud J, Mas-Coma S. Phenotypic analysis of adults of *Fasciola hepatica*, *Fasciola gigantica* and intermediate forms from the endemic region of Gilan. Iran Parasitol Int. 2006; 55: 249-60.
5. Torgerson P, Claxton J. Epidemiology and control. In: Dalton JP, editors. Fasciolosis. Oxon, USA: CAB International; 1999.
6. Hurtrez-Boussès S, Meunier C, Durand P, Renaud F. Dynamics of host parasite interactions:

the example of population biology of the liver fluke (*Fasciola hepatica*). Microb Infect. 2001; 3: 841-9.
7. Georgieva K, Georgieva S, Mizinska Y, Stoitsova S. *Fasciola hepatica* miracidia: Lectin binding and stimulation of in vitro miracidium-to-sporocyst transformation. Acta Parasitol. 2012; 57(1): 46-52.
8. Maldonado JA, Vieira GO, Garcia JS, Rey L, Lanfredi RM. Biological aspects of a new isolate of *Echinostoma paraensei* (Trematoda: Echinostomatidae): susceptibility of sympatric snails and the natural vertebrate host. Parasitol Res. 2001; 87: 853-9.
9. Faltýnková A, Nasincová V, Kablásková L. Larval trematodes (Digenea) of the great pond snail, *Lymnaea stagnalis* (L.), (Gasropoda, Pulmonata) in central Europe: A survey of species and key to their identification. Parasite. 2007; 14: 39-51.
10. Al-Habbib WMS. The effect of constant and changing temperatures on the development of the larval stages of *Fasciola hepatica* (L.) [PhD dissertation]. Dublin University, Ireland; 1977.
11. Rowcliffe SA, Ollerenshaw CB. Observations on the bionomics of the egg of *Fasciola hepatica*. Ann Trop Med Parasitol. 1960; 54: 172-81.
12. Al-Jibouri M, Hassan R, Al-Mayah H. Ecological factors affecting on eggs development and life span of miracidia of *Fasciola gigantica*. Kerbala J Pharma Sci. 2010; 1:70-4.
13. Ono Y, Isoda M. Studies on fascioliasis. Observation on the life history of *F. hepatica*. Jap Vet Sci. 1951; 13:87-96.
14. Hussein AA, Hassan IM, Khalifa RMA. Development and hatching mechanism of *Fasciola* eggs, light and scanning electron microscopic studies. Saudi J Biol Sci. 2010; 17: 247-51.
15. Imani Baran A, Yakhchali M, Malekzadeh-Viayeh, R. A study on geographical distribution and diversity of Lymnaeidae snails in West Azarbaijan Province, Iran. Pajouhesh and Sazandegi. 2010; 82: 53-63 (Persian)
16. Wilson RA. The hatching mechanism of the egg of *Fasciola hepatica* Linnaeus. Parasitol. 1968; 58:79-89.
17. Islam MN, Port GR, Mclachlan AJ. The biology of *Lymnaea peregra* (Muller) (Gastropoda: Pulmonata: Basommatophora) with special reference to the effects of herbicides on its reproduction. J Biol Sci. 2001; 1(6):532-40.
18. Harman WN, Berg CO. The freshwater Gastropoda of central New York with illustrated keys to the genera and species. Entomol (Ithaca). 1971; 1:1-68.
19. Calow P. On the regulatory nature of individual growth: some observations from freshwater snails. J Zool. 1973; 170:415-28.
20. De Francesco CG, Isla FI. Distribution and abundance of hydroid snails in a mixed estuary and a coastal lagoon, Argentina. Estuaries. 2003; 26:790-7.
21. Karimi GR, Derakhshanfar M, Paykari H. Population Density, Trematodal Infection and Ecology of *Lymnaea* Snails in Shadegan. Arch Razi Institute. 2004; 58:125-9.
22. Mas-Coma S, Bargues MD, Valero MA. Fascioliasis and other plant-born trematod zoonoses. Int J Parasitol. 2005; 35:1255-78.