Dynamics of haemocytes from *Pseudosuccinea columella* circulating infected by *Fasciola hepatica*

Dinâmica de hemócitos circulantes de *Pseudosuccinea columella* infectados por *Fasciola hepatica*

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Abstract

The lymnaeids are important in the epidemiology of *Fasciola hepatica*, a neglected and endemic zoonosis. The interaction between the internal defense system of *Pseudosuccinea columella* and *F. hepatica* has been little studied. In the present study the effect of infection by *F. hepatica* on *P. columella* circulating haemocytes was investigated. Changes in the average number of total circulating haemocytes have been observed at 30 minutes post-infection and 1, 7, 10, 14, 21, 28 and 50 days post-infection (dpi). Miracidia were observed head-foot and mantle at 30 minutes post-infection. Miracidia/Sporocysts in the mantle skirt 1 dpi, and fully formed sporocysts were observed in the head-foot at 7 dpi. Rediae became evident at 10 dpi and were located between the haemocoel and the muscles from 14 dpi; 50 dpi, the rediae in the digestive gland contained cercariae. The statistical analysis of the total haemocytes of *P. columella* infected by *F. hepatica* showed significant differences on the 30 minutes post-infection and 1, 14, 21, and 28 dpi in comparison to uninfected molluscs (0 dpi). Therefore, the interference observed on the internal defence system of *P. columella* may have direct association with the development of *F. hepatica*.

Keywords: *Fasciola hepatica*, haemocytes, *Pseudosuccinea columella*.

Resumo

Os limnaeideos são importantes na epidemiologia de *Fasciola hepatica*, uma zoonose negligenciada e endêmica. A interação entre o sistema interno de defesa de *Pseudosuccinea columella* e *F. hepatica* tem sido pouco estudada. No presente estudo, investigou-se o efeito da infecção por *F. hepatica* nos hemócitos circulantes de *P. columella*. Alterações no número médio de hemócitos circulantes foram observadas aos 30 minutos e 1, 7, 10, 14, 21, 28 e 50 dias após a infecção (dpi). Miracídios foram observados na região cefalopodal e manto aos 30 minutos após a infecção. Miracídio/esporocistos foram observados no colar do manto ao 1 dpi, e esporocistos totalmente formados na região cefalopodal aos 7 dpi. Rédias tornam-se evidentes aos 10 dpi entre a hemocele e músculos a partir de 14 dpi; e rédias com cercárias próximas a glândula digestiva aos 50 dpi. A análise estatística dos hemócitos totais de *P. columella* infectados por *F. hepatica* demonstrou diferenças significativas nos 30 minutos pós-infecção e 1, 14, 21 e 28 dpi em comparação aos moluscos não infectados (0 dpi). Portanto, a interferência observada no sistema de defesa interna de *P. columella* pode ter associação direta com o desenvolvimento de *F. hepatica*.

Palavras-chave: *Fasciola hepatica,* hemócitos, *Pseudosuccinea columella*.

Introduction

Fasciola hepatica (Trematoda: Digenea) is a parasite of hepatic ducts of several mammal species, including ruminants, rabbits, swine, rodents and humans, which are the definitive hosts, and lymnaeid snails act as intermediate host. This trematode exhibits a wide distribution, is important as a zoonotic parasite, and causes great financial loss in cattle breeding due mainly to liver condemnation (LIMA et al., 2009; VAN DER VOORT et al., 2013). *Pseudosuccinea columella* is the most frequent intermediate host of *F. hepatica* in the South and Southeast Brazil (COELHO et al., 2008). This is probably due to its high genetic variability, sympatric and allopatric association that may vary according to the origin of the snails and flukes involved (CARDOSO et al., 2006; COELHO et al., 2009).

Adult flukes settle into their definitive hosts hepatic ducts, and eggs are released into the external environment with the host faeces. In watercourses, miracidia develop within the eggs and hatch after 12-14 days under favourable light, temperature and humidity conditions. Propelled by their cilia, miracidia then swim in search of a lymnaeid, penetrate the host using cytolytic substance actions, and transform into sporocysts, which, in turn, yield the rediae. Several cercariae develop within the rediae and then exit the mollusc and encyst upon contact with a substrate, such as aquatic vegetation, to convert into metacercariae, the infectious form. The definitive host is infected through ingesting metacercariae.

Studies on the interaction between *F. hepatica* and lymnaeids are limited due to several factors involving both the parasite and intermediate host. Despite the high specificity between molluscs and trematodes, immunobiological studies between *F. hepatica* and lymnaeids are little explored. Nevertheless, the recognition of the trematode by the mollusc's internal defense system, made by haemocytes and soluble factors present in the haemolymph, from the penetration of miracidium to the transformation of sporocyst followed by the formation of rediae until the release of cercariae, may resemble the associations reported for other digenetic trematodes and gastropod molluscs (PEREIRA et al., 2008; CARON et al., 2014; COUSTAU et al., 2015). Fluctuations in the number of *Lymnaea stagnalis* haemocytes in the haemolymph during infection by *Trichobilharzia ocellata* have been observed (VAN DER KNAAP et al., 1987; AMEN et al., 1991). Other factors that also affect the success of the intra-mollusc trematode are age, strain or geographical origin of the lymnaeids (GUTIÉRREZ et al., 2011; COELHO et al., 2009; DREYFUSS et al., 2012). When infected, lymnaeids change biology which may reflect on the survival rate (MENDES et al., 2008; VIGNOLES et al., 2015).

Thus, new research on the interaction between the internal defense system of *P. columella* and *F. hepatica* is important because any insight can aid in understanding this association that has been poorly explored in the literature. Additionally, fascioliasis is a neglected, endemic and expanding disease in several regions of the world, and because molluscs are the first hosts for this parasite, studies in this field might aid in discovering control alternatives for this parasitosis. Therefore the present study evaluated the course of infection by *F. hepatica* through the quantification of the circulating haemocytes and the histological analysis of the *P. columella* of the municipality of Confins, Minas Gerais, Brazil lineage.

Materials and Methods

Mollusc source

Specimens of the parental *P. columella* generation were collected in an oxbow lake of the Mata river (19°40'50.34"S; 43°58 '23.42"W) in the municipality of Confins, Minas Gerais state, Brazil. In the laboratory, the specimens were fed lettuce leaves and maintained at a mean temperature of 24 °C in plastic tanks with water and polystyrene fragments to generate the spawn that gave rise to the specimens used during the experiment after reaching a mean size of 4-8 mm.

Miracidia and Pseudosuccinea columella infection

Fasciola hepatica eggs were obtained from faeces of infected sheep, the experiment was approved by the Ethics Committee the Federal University of Minas Gerais (CEUA/UFMG), under protocol number 18/2016. The eggs were transferred to Petri dishes containing dechlorinated water and incubated at 27 °C for 12 days to develop miracidia (MENDES et al., 2008). After hatching, two miracidia per host specimen were collected with a Pasteur pipette and transferred to a polystyrene plate (FALCON 3047) containing 2 ml of water for individual infection of each of the *P. columella* specimens with a mean shell length of 4-8 mm. The plates were covered and exposed to a 60 W light bulb at a distance of 50 cm for 24 h. After that, each well was analysed under a stereoscopic microscope at 40 X magnification to confirm the infection. The molluscs were maintained in plastic containers at a average room temperature of 24 °C and fed lettuce until they were used for the below described protocols.

Haemolymph collection and quantification of haemocytes of Pseudosuccinea columella infected by Fasciola hepatica

The molluscs were anesthetized 30 minutes post-infection and 0, 1, 7, 10,14, 21, 28, 50 dpi, by immersion in a Sodic Pentobarbital solution (Hypnol-Cristalia) at 0.4 mg/ml diluted in CBSS - Chernin 's Balanced Salt Solution (47.7 mM of NaCl, 2.0 mM of KCl, 0.49 mM of Na_2HPO_4 anhydrous, 1.8 mM of $MgSO_4$. $7H_2O$, 3.6 mM of $CaCl_2$.2 H_2O, 0.59 mM of $NaHCO_3$, 5.5 mM of glucose and 3 mM trehalose, pH 7.2) for 4 hours at room temperature, modified from Martins-Sousa et al. (2001), once the anesthetic was diluted in CBSS. The shells were then cleaned with absorbent paper soaked in 70% alcohol. A heparinized capillary tube of microhematocrit (Glasscyto -, 80UI/ml sodium heparin, 75 mm long x 1.6 mm external diameter) was inserted in circular motions in the head-foot region of *P. columella* until the muscles were transposed and the mollusc's body cavities were reached. Then, the extravasated haemolymph (5-20µl/mollusc) was collected by capillarity and transferred to a plastic film in an ice bath. Snail hemolymph was collected from individual snails, using 15 animals/group/dpi, was divided into 5 pools each consisting of 3 molluscs. Each pool was individually analysed.

After a 1:10 dilution in CBSS/citrate/EDTA solution supplemented by 100 U/ml penicillin, 100 µg/ml streptomycin (Sigma, St. Louis MO, USA), containing 4% Trypan Blue vital dye (Sigma, St. Louis MO, USA), the viability of the haemocyte population was estimated in 10 µl of total haemolymph diluted 1:10 in CBSS buffer containing 0.4% Trypan Blue (Sigma, St. Louis MO, USA). Membrane-damaged haemocytes allow the fast penetration of Trypan Blue and these blue cells were immediately counted in a Neubauer's chamber (MARTINS-SOUZA et al., 2003; PEREIRA et al., 2008). Three independent experiments were performed. Totalling 405 molluscs at the end of the experiment.

Histological analysis of Pseudosuccinea columella infected by Fasciola hepatica

For the histological evaluation, five *P. columella* specimens were used for each of the following intervals: 30 minutes post-infection, 0, 1, 7, 10, 14, 21, 28, 50 dpi, totalling 45 molluscs. The molluscs were immersed in Bouin's fluid for 24 hours for fixation. The mollusc tissues were then separated from the shells, dehydrated in a series of alcohol baths, cleared using xylol, and embedded in paraffin. Serial histological 5-7μm thick sections were mounted onto slides, stained with haematoxylin-eosin, and examined under an Olympus BX41N microscope coupled to an Olympus DP12 digital camera.

Survival curve of Pseudosuccinea columella infected by Fasciola hepatica

In order to analyse the survival curve, 70 infected *P. columella* and 70 uninfected *P. columella* were kept in tanks under the conditions described above. The tanks were monitored daily for 50 days to quantify the dead molluscs.

Statistical analysis

Statistical analysis was performed in the Graphpad Prism 5.0 software. The average number of the circulating haemocytes counts was compared between *F. hepatica* uninfected and infected *P. columella*, through the one-way ANOVA test followed by Tukey's post-test. Kaplan-Meier Survival Curves and the Log-Rank test were used to analyse mollusc survival.

Results

Haemolymph collection and quantification of haemocytes of Pseudosuccinea columella infected by Fasciola hepatica

Most the experimental period, the average number of total haemocytes present in the haemolymph was lower in the infected molluscs compared to uninfected specimens.

Such variations in the number of total haemocytes showed statistically significant differences in the 30 minutes post-infection, 14, 21 and 28 dpi intervals (Figure 1, Table 1).

Oscillations of haemocytes number were observed between 30 minutes post-infection and 7 dpi, when miracidium transformation into sporocyst occurred. At 30 minutes post-infection ($p<0.05$), a sudden dilution in the number of circulating cells was observed, contrary to what happened at 1 dpi ($p<0.05$). Between 7 and 10 dpi, the levels are similar to those observed in uninfected molluscs, an interval when free rediae were observed in the molluscs tissues. Reduced levels of circulating haemocytes also occurred among 14 ($p<0.001$), 21 ($p<0.05$) and 28 ($p<0.05$) dpi in the haemolymph, a period when rediae were observed migrating through the haemocoel

and *P. columella* tissues. At 50 dpi, however, a quantity of haemocytes was similar to that observed in uninfected molluscs (Figure 1).

In most the period under study, the average number of dead cells was low. Only on 1 and 7dpi the total haemocytes increase, coincided with an increase in the number of dead cells (Figure 1b).

Histological analysis of Pseudosuccinea columella infected by Fasciola hepatica

At 30 minutes post-infection, *F. hepatica* miracidia covered with remnant ciliated plates were observed in the connective tissue of the *P. columella* head-foot and mantle regions. Lacunae were observed around the miracidia in these regions, and ruptured muscle fibres were observed in the transverse and longitudinal planes (Figure 2a).

At 1 dpi, sac-shaped sporocyst containing round translucent cells were observed in the *P. columella* mantle skirt. The first evidence of ruptured connective tissue and lacunae formation around developing sporocysts. (Figure 2b).
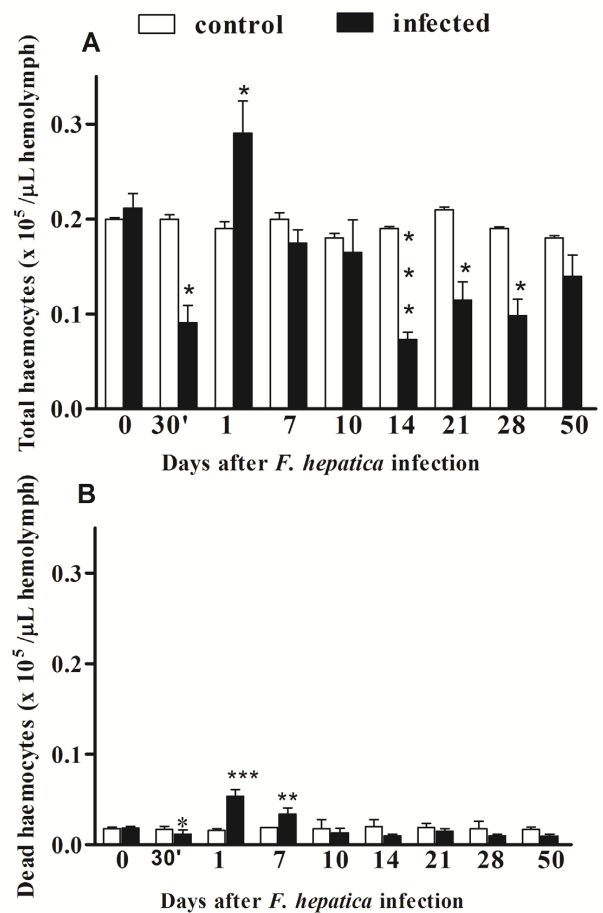

Figure 1. Total and dead circulating haemocytes during the development of *Fasciola hepatica* in *Pseudosuccinea columella*. Asterisks represent * ($p<0.05$) ** ($p<0.01$), *** ($p<0.001$) the statistical difference between the number of haemocytes present in infected molluscs compared to uninfected ones.

Table 1. Number of total and dead cells in hemolymph of *Pseudosuccinea columella* infected with *Fasciola hepatica*. Each point represent the average ±SD and the experiment was repeated three times with similar results.

Interval of Infection	Total cells (x10^5/µL)		Dead cells (x10^5/µL)	
	Infected	Control	Infected	Control
30' pos infection	0.090 ± 0.055[a]	0.090 ± 0.055	0.011 ± 0.011[a]	0.010 ± 0.017
1°	0.290 ± 0.183[a]	0.290 ± 0.183	0.053 ± 0.038[c]	0.016 ± 0.01
7 °	0.174 ± 0.064	0.174 ± 0.064	0.033 ± 0.031[b]	0.019 ± 0.00
10°	0.165 ± 0.103	0.165 ± 0.103	0.013 ± 0.014	0.018 ± 0.03
14°	0.073 ±0.044[c]	0.073 ± 0.044	0.009 ± 0.011	0.020 ± 0.04
21°	0.114 ± 0.087[a]	0.114 ± 0.087	0.015 ± 0.011	0.019 ± 0.020
28°	0.098 ± 0.066[a]	0.098 ± 0.066	0.010 ± 0.006	0.018 ± 0.03
50°	0.139 ± 0.087	0.139 ± 0.087	0.009 ± 0.009	0.017 ± 0.010

The letter "a" represents p <0.05, the letter "b" represents p <0.01, the letter "c" represents p <0.001, which are statistical differences between the number of hemocytes of the infected group in relation to the group not infected.
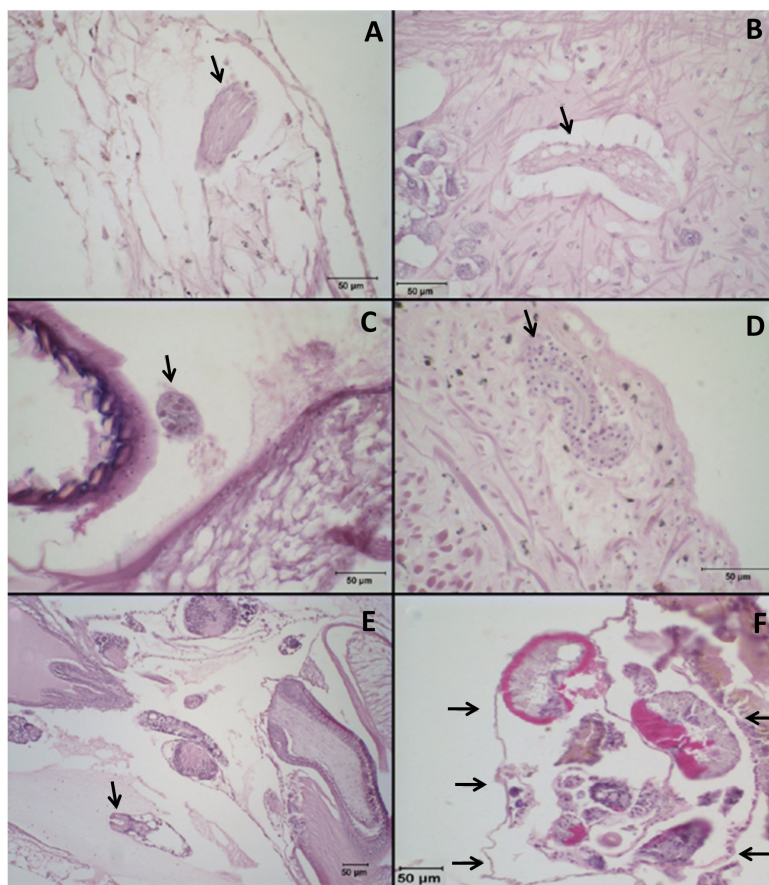

Figure 2. Histology of *Pseudosuccinea columella* infected by *Fasciola hepatica*. (A) Arrow: Miracidium at 30 minutes post-infection forming a gap at the mantle; (B) Arrow: Miracidium/Sporocysts head-foot region 1 dpi; (C) Arrow: Sporocysts near the radula at 7 dpi; (D) Arrow: Rediae at 10 dpi in the head-foot region; (E) Arrow: Rediae at 14 dpi in the haemocele and it is shown inset; (F) Arrows: Redia at 50 dpi in the digestive gland harbouring cercariae.

At 7 dpi, round sporocysts were present in the *P. columella* head-foot region near the host. The outer muscle layer of these larvae was developed and surrounded germinal cells with intense basophilia, which would eventually develop into rediae radula (Figure 2c).

At 10 dpi, developing *F. hepatica* rediae were observed in the *P. columella* head-foot region and exhibited a pharynx as well as a caecum. This larval stage was present in lacunae of ruptured mollusc connective tissue, as well as cellular reactions were discrete (Figure 2d).

At 14 dpi, rediae in different developmental stages were free in the haemocoel (Figure 2e). Certain such rediae exhibited cells that would give rise to the tegument, pharynx, caecum and germinal cells, and other rediae exhibited the above structures. In the mollusc haemocoel, containing hemocytes was observed in the haemolymph around the rediae (Figure 2e). Rediae migration

Life cycle of *Fasciola hepatica* in *Pseudosuccinea columella*
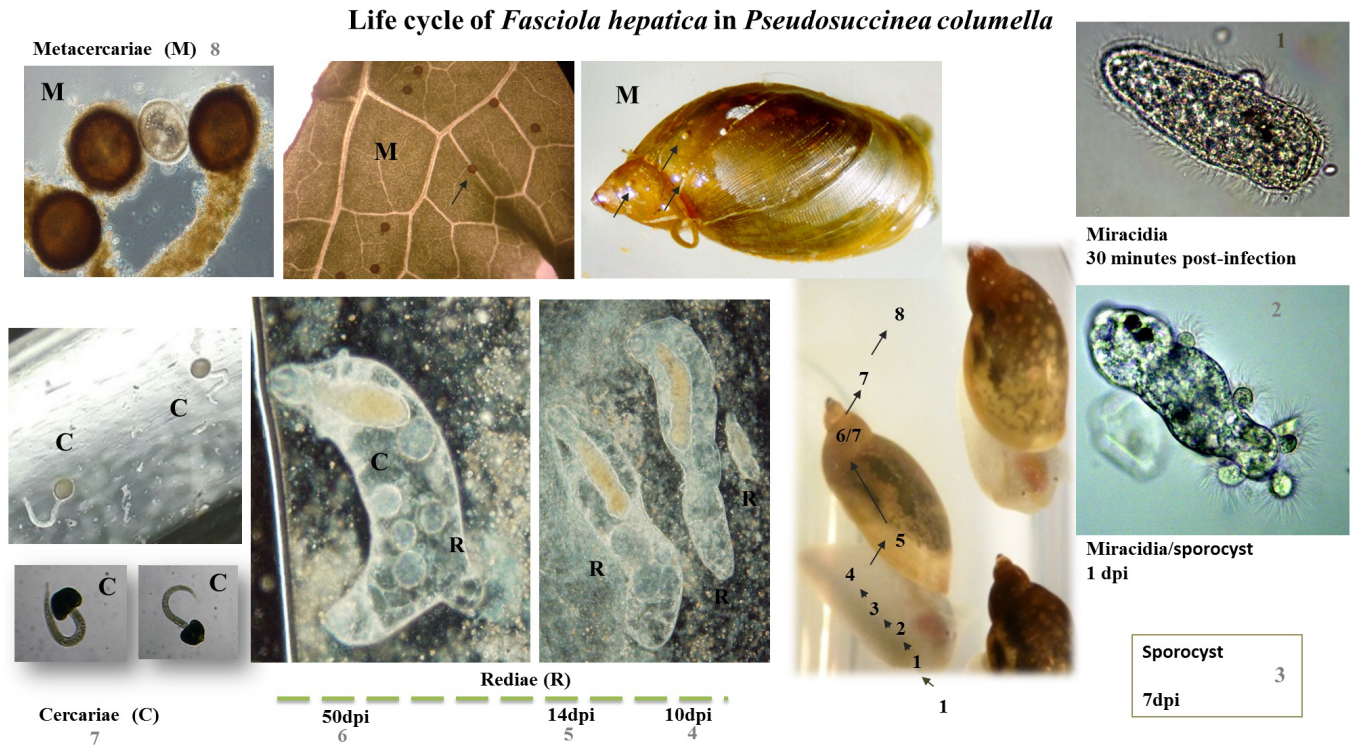
Figure 3. Schematic representation of the life cycle the *Fasciola hepatica* in *Pseudosuccinea columella* (1) Miracidium: 30 minutes post-infection → (2) Miracidium/sporocyst: 1dpi → (3) Sporocyst: 7 dpi→ (4, 5, 6) Rediae: 10,14,50dpi→(7) cercariae: 50 dpi→(8) metacercariae: 50 dpi.
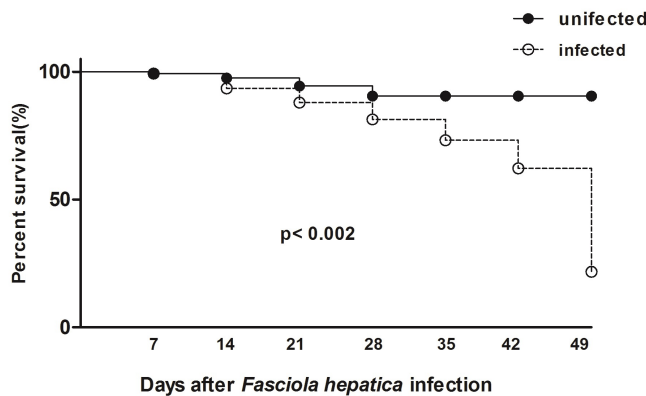
Figure 4. Survival curve of *Pseudosuccinea columella* infected by *Fasciola hepatica*. Statistically significant differences comparing between survival curve of the infected group was statistically different than the survival curve of uninfected group *p* < 0.002.

happened until 28 dpi with a similar pattern to 14 dpi (data not shown).

At 50 dpi, the digestive glands exhibited rediae harbouring daughter rediae and developing cercariae as well as disorganised epithelial and connective tissue structures in the digestive gland lobules due to the parasite (Figure 2f). Both developing and fully developed cercariae were also observed within the rediae at 50 dpi. On this interval, presence of metacercariae adhered to the experimental tank was also observe, see also the scheme the life cycle of *Fasciola hepatica* in *Pseudosuccinea columella* (Figure 3)

Survival curve of *Pseudosuccinea columella* infected by *Fasciola hepatica*

Differences between the infected and uninfected molluscs survival curves were observed during the experiment (*p* <0.002). In the infected group, the pattern of mortality increased from the 14dpi. At the end of the 28 dpi, 20% of the infected molluscs died, and between the 35 and 42 dpi plus 20% infected molluscs died. At the end of the experiment, only 20% of the infected molluscs were alive (Figure 4), while uninfected 92.5% survived.

Discussion

This study showed that the population of *P. columella* from Confins-MG is susceptible to *F. hepatica*, as reported for other populations of Brazilian *P. columella* from the Ribeira and Paraíba valleys, in the state of São Paulo (UETA 1980a, b; AMATO et al., 1986; OLIVEIRA et al., 2002), and from the meso-region of southern Minas Gerais and Pelotas, in the south of the country (COELHO et al., 2009). Recently, Dracz & Lima (2014) observed the spontaneous elimination of *F. hepatica* cercariae by this gastropod, from the municipalities São José da Lapa, and Pedro Leopoldo-MG.

Studies evaluating changes in the internal defence system of *P. columella* during the development of *F. hepatica* in Brazilian molluscs are however scarce. The asexual multiplication process of the mollusc could induce changes in the average number of

total circulating haemocytes of the infected molluscs, compared to uninfected ones.

At 30 minutes post-infection, there was a decrease of approximately twice the average total number of circulating haemocytes of *P. columella* in relation to uninfected molluscs, and yet no striking cellular infiltrate was observed close to the newly-penetrated miracidia in the molluscs. Van der Knaap et al. (1987) suggested that the decrease of the number of circulating haemocytes em *L. stagnalis* would have a direct association with the mobilization of these cells to the sites where miracidia penetrate induced of *T. ocellata*. Adema et al. (1994) observed that 30 minutes after *in vitro* interaction, haemocytes of different molluscs present different *Echinostoma paraensei* larval forms recognition patterns. This recognition pattern may be associated with the specificity of the mollusc-helminth interactions (PEREIRA et al., 2008). It should also be noted that the mollusc innate immune system, comprised of haemocytes and hemolymph soluble factors can recognize not only the parasite and excreted-secreted products (ES), but also the induced inflammation process (COUSTAU et al., 2015) a fact not demonstrated in this study.

At 1 dpi, the population of circulating haemocytes of *P. columella* increased to levels similar to those observed for the uninfected molluscs (p<0.05). At 1 dpi, there was a loss of the ciliated plates of the miracidia of *F. hepatica* and the transformation into sporocysts occurred (DIAWARA et al., 2003). The authors Van der Knaap et al. (1987) demonstrated in *L. stagnalis* infected with *T. ocellata*, the increase phagocytic index of after haemocytes 24 hours of infection by the trematode. They attributed the possibility of haemocyte activation to the loosening of the miracidia ciliated plates, which would be phagocytized along with cellular debris from damaged tissues of the mollusc, during penetration of miracidia and development of parental sporocysts. This hypothesis can be corroborated by Gourbal et al. (2008) who reported, through proteomic studies of *in vitro* transformed sporocysts of *F. hepatica* that, after 24 hours, the ES may interfere with the recognition of haemocytes present in the mollusc haemolymph parasitized with the trematode. At 7 dpi, *F. hepatica* developed sporocysts in *P. columella* were observed, when the total average number of circulating haemocyte was restablished in the haemolymph, reaching values similar to those of uninfected molluscs. By histology, sporocysts near the radula were found. According to Humphries & Yoshino (2003), the elimination of metazoan parasites, such as trematodes, by molluscs would be directly associated with the formation of multicellular capsules of haemocytes, in a coordinated action with soluble factors of the haemolymph. In the *Biomphalaria* spp. resistant to the trematode, after migration of haemocytes to the vicinity of the sporocyst, tegument was noted and there was also the release of products that destroyed and killed the parasite by cytotoxicity (PEREIRA et al., 2008), a fact not demonstrated in this study.

Beginning at 10 dpi, rediae were observed developing within lacunae of ruptured connective tissue in the *P. columella* head-foot region. Rediae were also observed in the haemocoel at 14 dpi. In the present study, *P. columella* tissue lesions caused by rediae were similar to the lesions observed by Diawara et al. (2003) in *Galba truncatula* that were naturally infected by *F. hepatica*. Despite the fact that during this infection interval, the parasite is

more developed, the total circulating haemocyte profile is similar to that between 7 dpi and uninfected molluscs. From 14 dpi on, the average number of circulating haemocytes reduced three times in the circulating haemolymph, period when rediae migrated through the hemocoel towards the digestive gland of the mollusc. According to Yoshino et al. (2001), during the intra-mollusc development of trematodes, the interaction interface between the parasite and the intermediate host would be a dynamic medium, made up of cell membranes and non-cellular components of the mollusc, as well as the parasite tegument. These authors reported that in susceptible molluscs, the haemocytes were not linked to the sporocysts tegument due to the release of ES, camouflaging the parasite's internal defence system of the invertebrate host, in a similar way to what was observed in serial histological sections of this study at 14 dpi. Loker & Hertel (1987) reported the approximation of hemocytes at 15 dpi when *E. paraensei* rediae was present in *B. glabrata* aorta and the number of haemocytes in the circulation was greater than that of the uninfected mollusc group. This contrasted with the results of this study, where there was a reduction of circulating haemocytes of *P. columella* during the release of the *F. hepatica* rediae, which may be related to the susceptibility of the species of lymnaeid to the development of the parasite.

In this study, however, from 10 dpi on, *F. hepatica* rediae in the histological sections were observed, and the total number of circulating haemocytes in the haemolymph decreased and increased again at 50 dpi. According to Rondelaud et al. (2009), there may be 3 or 4 generations of *F. hepatica* rediae occurring from the 7 dpi on the same *Galba truncatula* specimen, and the first generation of rediae produced daughter rediae, which in turn originated cercariae and thus the development of the rediae would occur normally, with no death of mother rediae in the first or second week of the infection. These authors observed that the daughter rediae could be released by the mother rediae in the same period in which the latter would be emerging from the sporocysts. This may explain the morphological differences between the rediae in the histological sections and the ones in the *P. columella* circulating haemocyte counts throughout the course of infection by *F. hepatica* in this study observed from 10 to 50 dpi.

Contrary to what was observed in this study, Van der Knaap et al. (1987) reported in *L. stagnalis-T. ocellata* the increase in the number of circulating haemocytes in every studied interval, from 7 to 91 dpi, period when the intramollusc transformation of miracidia into sporocysts and the release of cercariae. According to them, the proliferation of haemocytes was due to the recruitment of connective tissue haemocytes to the haemolymph of the molluscs, due to the fact that this was not observed *in vitro*. According to these authors, the haemocyte kinetics of trematode infected molluscs may be modulated during infection, depending on the combinations between host and parasite, as well as the experimental conditions. On the other hand, Amen et al. (1991) followed the development of *T. ocellata* in *L. stagnalis* through haemocyte count at 0, 14, 28, 42 and 56 dpi and histological analysis of the infected molluscs in the 2, 4, 6 and 7 wpi. These authors reported an increase in the number of haemocytes during the development of the parasite, of sporocysts at 14 dpi migrating from the head-foot to the digestive gland at 28 dpi, when this cell

population decreased. An increase in the number of haemocytes also occurred between 42 and 56 dpi, intervals where sporocysts and early release of cercariae occurred. At 56 dpi, the number of circulating haemocytes was larger.

The percentage of dead haemocytes ranged (16-18%) and although in this work was not tested the ES participation on the interaction between *F. hepatica* and *P. columella*, further studies are needed to understand the number the of dead cells found.

As for the average number of *P. columella* dead cells, it was statistically significant (p <0.01) between the 30 minutes post-infection intervals and the uninfected molluscs. In the intervals between 1 and 7 dpi, the percentage of dead cells ranged from 16 and 18% and were similar to the viability levels accepted in the literature, as reported by Adema et al. (1994) in *L. stagnalis* haemocyte viability tests performed with trypan blue. A percentage of dead cells ranging between 19 and 11% was considered satisfactory, when the cells of these gastropods were recovered.

During an experimental infection of *Lymnaea* spp. by *F. hepatica*, parasite-host competition for energy reserves in the digestive glands began at 20 dpi and intensified until 60 dpi, which promoted cell death in this gland (HUMICZEWSKA, 2000), reflecting on the mortality rate observed in infected molluscs, which happened in this study.

Conclusion

In this study, the interference observed on the internal defence system of *P. columella* may have a direct association with the development of *F. hepatica*, but future studies are needed to characterize the interaction of this host-parasite relationship.

Acknowledgements

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