RESEARCH NOTE

Effects of the Digenea *Proctoeces lintoni* (Fellodistomidae) in the Proportion of Hemolymphatic Cells in *Fissurella crassa* (Mollusca: Archaeogastropoda)

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The progenetic digenea *Proctoeces lintoni* Siddiqi & Cable, 1960 (Fellodistomidae) is a common parasite in the gonads of key-hole limpets of the genus *Fissurella* (Archaeogastropoda). This helminth causes partial castration in *Fissurella crassa* (ME Oliva 1992 *Mem Inst Oswaldo Cruz 87*: 34-42) and fecundity (expressed as egg/mm shell) of infected individuals is dramatically reduced when intensity of infection increases (ME Oliva & AA Vega 1994 *Mem Inst Oswaldo Cruz 89*: 225). These effects may strongly affect the populations due to decreased fecundity. Despite the impact of the helminth, no host response seems to occur.

To examine the possibility that primary cellular reactions against the helminths could be developed, here we analyze changes in the proportion of hemocytes (granulocytes and hyalinocytes) in infected and non-infected key hole limpet *F. crassa*, obtained from the intertidal zone of Caleta Constitución, northern Chile (23°25'S 70°35´W). Live key-hole limpets were brought to the laboratory and kept in 60 l glass containers at room temperature. A total number of 83 specimens (ranging 23.2 to 78.8 mm total length) were collected and measured (to the nearest 0.1 mm) and their shells were carefully rinsed with distilled water to remove salts, after which all the specimens were dissected, sexed, and presence of parasites was quantified. Thirty non-infected and 30 infected (1 to 27 parasites) key hole limpet, in the range of 30 to 60 mm in total length, were selected for the analysis. Hemolymph was extracted directly from the heart with a syringe after cardiac punction. At least 100 µl of hemolymph of individual key hole limpets was centrifuged (5000 G * 15 min at 4°C) and smear were prepared on glass standard slides. Then the slides were stained with May Grunwald-Giemsa and at least 200 cells were counted to determine the relative proportion of each one of the two cellular types, hyalinocytes and granulocytes, identifiable in according to MA Barracco et al. (1993 *Mem Inst Oswaldo Cruz 88*: 73-83). Key-hole limpets were thus assigned into three categories: non infected, low infection (1 to 9 parasites, average = 4.4, SD = 2.6, n = 14) and strong infection (> 10 parasites, average 16.3, SD = 5.1, n = 16). Mean size of key hole limpets (Table) differed significantly among the categories ($F_{2,57} = 59.7$, $P < 0.001$), but proportion of granulocytes (after arcsine transformation) is not affected by host size ($r = 0.015$, $P = 0.937$, $n = 30$). One-way ANOVA applied to arcsine-transformed data of proportion of granulocytes in healthy males and females revealed non significant differences between the sexes (males 66.4%, females 66.8%, $F_{1,28} = 0.146$, $P = 0.705$). Thus we concluded that both, host size and host sex do not affect the proportion of granulocytes. Proportion of hyalinocytes and granulocytes in non-infected, low infected and strong infected mollusks is shown in the Table. A one-way ANOVA shows that proportion of granulocytes differed significantly among the above categories ($F_{2,57} = 58.3$, $P = 0.001$). Further, the *a posteriori* Tukey test showed that proportion of granulocytes in low infected and strong infected key-hole limpets do not differ significantly, but both were different from the non-infected, suggesting that changes in the proportion of cells is due to the presence of the parasite and the intensity of infection (in the sence of AO Bush et al. 1997 *J Parasitol 83*: 575-583) do not affect the cellular response.

As pointed by Barracco (*loc. cit.*) the immunobiology of the trematode-host relationships is strongly associated with the circulating cells in the hemolymph and with humoral factors in the host plasma. Phagocytosis and encapsulation have been often reported as primary defense mechanisms of mollusks against foreign substances (SE Ford 1986 *J Invertebr Pathol 47*: 283-294). A particular characteristic of the infection originated by *P. lintoni* in *F. crassa* is the absence of encapsulation. Encapsulation have been defined as the most common

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TABLE

Mean size (standard deviation in parenthesis), proportion of hyalinocytes and granulocytes in non-infected, low infected (1-9 parasites) and strongly infected (> 10 parasites) key-hole limpets *Fissurella crassa*

	Mean size	Hyalinocytes	Granulocytes
Noninfected	39.0 (5.0)	16.0	84.0
1 - 9 parasites	49.9 (7.1)	6.6	93.9
> 10 parasites	58.2 (6.2)	5.7	94.3

defensive mechanism against foreign material in mollusks (TC Cheng 1967 *Adv Mar Biol 5*: 1-424). In this work, we did not detect a clear and effective cellular defensive mechanisms. Even though an increased proportion of phagocitary cells (granulocytes) is evident, suggesting the existence of chemotaxis as the initial stage of encapsulation, the whole process of encapsulation is not completed. Absence of such a primary responses may be independent of the recognition of the parasite as a foreign material, although this may be true, there might be factors that inhibit the synthesis of membrane receptors, as suggested by WS Fisher [1988 *Am Fish Soc Special Pub 18* (315): 225-237], who points out that parasites can override membrane receptors of hemocytes. Absence of an effective primary immune response can strongly affect the reproductive success of key-hole limpets. The loss of reproductive potential in *F. crassa* may reach up to 47% in specimens with high infection (Oliva & Vega *loc.cit.*) and a similar consequence is evident for *F. limbata* infected by *P. lintoni*, showing a loss of fecundity that reach 35.3% in average (ME Oliva 1993 *Acta Parasitol 38*: 155-156). *Fissurella* spp. are important food sources in northern and central Chile, and the synergistic effect of the impact of the parasite on fecundity, the absence of primary immune responses along with increased overexploitation can altogether make this species highly vulnerable to population decrease.