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GAMMA ^{60}Co $DL_{50/30}$ OF *Biomphalaria glabrata* (SAY, 1818)

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SUMMARY

The variation of resistance to ^{60}Co gamma-rays of *Biomphalaria glabrata* was studied. A population of 480 mollusks was observed during 30 days - distributed in 8 groups of snails isolated and 8 groups of snails in colonies - after exposure (30 snails per group per dose) to increasing doses of gamma radiation. Doses of 10, 20, 40, 60, 80, 160, 320 and 640 Gy from a Gamma-cell ^{60}Co irradiator, were applied to the test groups and two groups control (non-irradiated) of snails – isolated and colony - were kept apart. After have been exposed, the snails were drew back to the aquaria where they were maintained before. The survival was estimated on a daily score of the alive animals in each group-dose, starting after the irradiation exposure day. As a result, the survival self-fertilization forms ($DL_{50/30}$ = 218.2 Gy) was found greater than in cross-fecundation forms. These data point to a low radio-resistance on the cross-fertilization forms - the sexual reproductive form - which is most found in nature. The lower radio-resistance of the cross-fertilization forms suggests the presence of some sex-linked hormonal factor related to this phenomenon.

KEYWORDS: Lethal dose; *Biomphalaria glabrata*; Gamma radiation; *Schistosoma mansoni*; Radio-resistance.

INTRODUCTION

Biomphalaria glabrata is a vector of schistosomiasis in Brazil, common in the northern and northeastern region on creeks, lakes and rivers[9]. It can change to a self-fertilization form when the environmental conditions are severe but normally its reproductive form is cross-fertilization and they live in colonies[13]. Schistosomiasis, caused by the worm *Schistosoma mansoni* (SM) is a disease widespread in tropical regions and endemic in 74 countries, infecting more than 200 millions people that endures intestinal bleeding, hepatic fibrosis and spleen complications. Migrations of infected people from endemic areas and the dispersion of the intermediary vectors are related to its expansion[12]. Other vector of this disease in Brazil is the *B. straminea* (Dunker-1818) having greater prevalence[12] in northern and north regions but having a lower fertility, as shown in a laboratory raised snails study[1]. The species *B. tenagophila*, with the lowest fecundity[14] among them, is the most common vector of schistosomiasis in southern and southeastern region of Brazil[9] .

Ionizing radiation of *B.glabrata* embryos resulted in substantial effects in its development[8]. Adult snails[2] raised in colonies – cross-fertilization forms – revealed a of 60 Gy $DL_{50/30}$ while isolated snails – self-fertilization forms – presented a 90 Gy $DL_{50/30}$[10]. Previous studies reported an attenuation of virulence after gamma irradiation of SM cercariae in mouse[15] and *B.glabrata*[6] infection.

In order to compare the ^{60}Co gamma-rays resistance between the self-fertilization and the cross-fertilization forms of the *Biomphalaria glabrata*, a protocol of study observed its $DL_{50/30}$ variation, which would help further investigations concerning schistosomiasis control.

MATERIAL AND METHODS

A population of 480 adult *Biomphalaria glabrata* snails reared in laboratory during 10 months, originated from the São Lourenço da Mata strain found in São Lourenço da Mata - PE, donated by Centro de Pesquisas Aggeu Magalhães - FIOCRUZ. Observed during 30 days, it was divided into 16 groups of 30 snails; 8 groups were kept under total reproductive isolation in individual aquaria and the 8 remaining groups were maintained in colonies in collective aquaria. The test groups were exposed to one dose of gamma radiation of 10, 20, 40, 80, 160, 320 and 640 Gy from a Gamma-cell ^{60}Co irradiator from Radionics Labs., with an actual radiation rate of 0.97 Gy/min. The 2 control groups (group-dose 0 Gy), having 30 isolated and 30 colonized snails, were placed in identical conditions as the endured by the snails from the irradiated groups, but did not receive any gamma irradiation.

For the irradiation exposure, the mollusks were carefully placed into dry glass tubes and the isolated snails had a thin layer of cotton separating each other (to avoid copulation). In order to minimize eventual differences induced by the darkness and humidity existing in the

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irradiation chamber, the control groups were kept in the dark during 5h and 24 min, time necessary to irradiate with half the maximal dose (320 Gy), in a dark environment at the same temperature and then placed back into aquaria.

After irradiation the mollusks were put back to the aquaria were they lived before, with artificial temperature and light cycles as proposed by MICHELSON[5]. The isolated groups had mini aquaria and the colonized groups the large ones (11.5 X 9.3 x 11.5 cm), maintaining ever the same 50ml water ratio per snail. The snails were fed "ad libitum" with lettuce (*Sativa lectura*) leaves. The water used was drinking tap water (pH 6.8 to 7.8) which was renewed daily.

The survival estimate was obtained by a daily count of the surviving animals in each group-dose, from the irradiation exposure and continued until one month.

RESULTS

The mollusks were observed during 29 days after the irradiation. The score of surviving snails, in each group-dose revealed to have an inverse relationship to the applied dose, as expected. The daily number of survivors was obtained subtracting the number of dead snails per dose from the population existing on the day before, resulting on the mortality per dose/day (Table 1).

Table 1
Values of the survivors and dead *B. glabrata* snails along 30 days, after ^{60}Co gamma-rays irradiation on colonies and isolated groups

Dose	Colony			Isolated		
Gy	Survivor	Dead	Dead%	Survivor	Dead	Dead%
0	26	4	13	27	3	10
10	19	11	37	22	8	27
20	26	4	13	23	7	23
40	25	5	17	24	6	20
80	4	26	87	26	4	13
160	1	29	97	23	7	23
320	0	30	100	1	29	97
640	0	30	100	1	29	97
Average	12.625	17.375	57.916	18.375	11.625	38.75

The percent normalization of data rendered easier the comparison between the different dose groups. Environment changes and mollusks manipulations during the irradiation procedures, must have stressed them and can be considered as a lethal factor. However, as this was a common damage, their effects cancel each other on control and test groups.

The mortality (Table 1) is displayed in the Figure 1, being the cross-fertilization forms mortality represented by a dotted line and that from the isolated groups, represented by a solid one. A darker horizontal straight line defines the limit of 50% of each group, that is, 15 snails. The $DL_{50/30}$ of ^{60}Co values obtained were 59 Gy for colonies and 218.2 Gy for sexually isolated, after a mathematical interpolation of data from table 1 in order to achieve the value of each crossover.

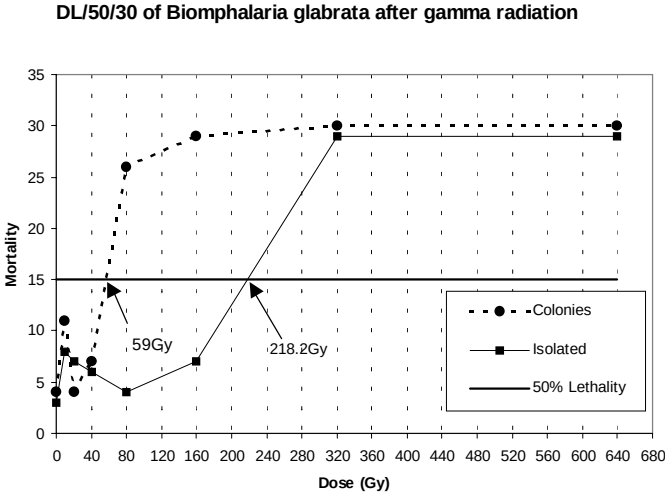

Fig. 1 - Line curves showing the lethality of isolated and colonized groups of *B. glabrata* snails, after ^{60}Co. Gamma-Rays irradiation, along 30 days. The centered darker line is the value of 50% of snails existing in each group. Arrows point the intersection of the 50% population line by the lethality curve from each group and gray vertical lines show its value over the X-axis.

DISCUSSION AND CONCLUSIONS

PERLOWAGORA-SZUMLEWICZ[10,11] in a study of $DL_{50/30}$ of *B. glabrata* after X-rays irradiation revealed a value around 60 Gy for self-fertilization and 90 Gy for the cross-fertilization forms. Similar results were found in the snail *Oncomelania formosana* - vector of *Shistosoma japonicum* - exposed to X-rays[3]. These studies used an analogous protocol and the lethal dose was estimated by the last crossing of each curve fit, along the doses applied, over the line of 50% of lethality (classical methodology).

However, X rays have a lower frequency energy spectrum as ionizing radiation than gamma radiation, used in the present work using the classical $DL_{/50/30}$ methodology. As a result it was found 90 Gy and 218.2 Gy as the $DL_{50/30}$ of respectively cross-fertilization and self-fertilization forms of *B. glabrata* (Figure1). Despite cross-fertilization *B. glabrata* be the reproductive form most found in nature[1], this finding suggest a lower resistance for it, when exposed to ionizing radiation.

The gamma irradiation - despite its lower energetic profile – seems to provoke a higher radio-resistance than X-rays on the self-fertilization forms, than gamma-rays. Table 1 shows the resulting mortality data and points to a lower radio-resistance among the cross-fertilization forms. So, it can be assumed that the difference between self-fertilization forms $DL_{50/30}$ after X-rays in the before cited work and gamma-rays irradiation is certainly due to the nature of the applied radiation.

LIARD *et al.*[4], noted that the whole *B. glabrata* group irradiated with gamma-rays dose of 320 and 640 Gy died after 60 days, but in a

previous work[7] it was found that all *B. straminea* snails died around the 11th to the 18th day under the same doses. This fact implies in a higher radio-resistance of *B. glabrata* as compared to *B. straminea.*

The average of all dead snails (see Table 1) revealed a self-fertilization forms $DL_{50/30}$ near 33% inferior to the cross-fertilization forms lethality, indicating a higher sensitivity to gamma-rays in the last groups. Moreover, the lower standard deviation found in self-fertilization forms data, suggests a more stable response against the ionizing aggression and an enhanced protective mechanism in this groups.

In conclusion, *B. glabrata* in self-fertilization form showed to be more radio-resistant to ^{60}Co gamma radiation than cross-fertilization form. This fact led to think of sexual hormonal interactions occurring in this last form. Also, the presence of some sensitizing organic or hormonal factor linked to the cross-fertilization reproductive form can induce the colonized snails to be more sensitive to radioactive aggressions. On the other hand, self-fertilization always occur under hostile environmental conditions[1] and can be seen as a resistant form of reproduction. Under this angle, it is natural the observed higher radio-resistance.

Biological radio-resonant interaction aspects on the energy level carried by Co60 gamma radiation will be studied in further works on *B. glabrata* hormonal and radio-protective mechanisms effects, envisaging to contribute to the epidemiological control of schistosomiasis.

RESUMO

$DL_{50/30}$ raios gama de ^{60}Co em *Biomphalaria glabrata* (Say, 1818)

A variação da resistência entre indivíduos em autofecundação e fecundação cruzada de *Biomphalaria glabrata* foram estudadas. Uma população de 480 moluscos foi observada durante 29 dias, distribuída em 8 grupos de caramujos isolados e 8 grupos em colônias após a exposição (30 caramujos por grupo-dose) a doses crescentes de radiação gama. Foram usadas doses de 10, 20, 40, 60, 80, 160, 320 e 640 Gy de um irradiador Gamma-Cell ^{60}Co. Dois grupos não irradiados – isolado e colônia – foram separados como controle e após a irradiação todos os caramujos voltaram para aquários onde viviam antes. A sobrevida foi estimada pela contagem diária dos animais vivos em cada grupo-dose, a partir do dia da irradiação.

O resultado mostrou maior sobrevivência nos grupos isolados ($DL_{50/30}$ = 218.2 Gy) que nos grupos colonizados ($DL_{50/30}$ = 59 Gy). Estes dados apontam para uma baixa radio-resistência nos grupos colonizados – com forma de reprodução sexuada – que são mais comuns na natureza. A baixa resistência observada sugere a presença de fatores humorais e hormônios sexuais ligados ao fenômeno

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