

# Evaluation of the effect of radioactive cobalt-60 and ultraviolet rays on *Giardia lamblia* infectivity to mice

Original  
Article

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## ABSTRACT

**Background:** *Giardia lamblia* is a flagellated unicellular eukaryotic micro-organism that commonly causes diarrheal disease worldwide. Although giardiasis is usually self-limited, it can develop into chronic and life-threatening disease. Most waterborne outbreaks (74.8%) were associated with drinking water as *Giardia* cysts are known to be resistant to chlorine at concentrations typically applied for water treatment.

**Objective:** To evaluate the effect of radioactive cobalt-60 and 254 nm ultraviolet (UV) irradiation on infectivity of *Giardia* cysts to mice.

**Material and Methods:** The study was conducted using 60 BALB/c mice divided into 6 groups with 10 mice in each. Group 1 received *Giardia* cysts treated with cobalt-60 (dose 0.25 KGy). Group 2 received cysts exposed to UV irradiation (wave length 254 nm). Groups 3-6 served as controls. Techniques used for evaluation of the infectivity of *Giardia* cysts included direct stool examination, duodenal aspiration with examination of the aspirate for the presence of *Giardia* cysts or trophozoites and histopathological examination of the small intestine of each mouse.

**Results:** Infectivity of *Giardia* cysts was reduced to 50% by experimental irradiation with cobalt-60 and 20% by UV, as shown by histopathological examination.

**Conclusion:** Low dose radioactive cobalt-60 and 254 nm UV radiations may be used as a control measure to prevent giardiasis, and as a mean of water treatment; but further studies are recommended for employment of both methods together or using smaller doses of each, thus benefitting from them both with less side effects.

**Key Words:** Cobalt-60, *Giardia lamblia*, histopathology, UV rays.

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## INTRODUCTION

*Giardia lamblia* is the most common intestinal protozoan parasite causing diarrhea in children especially those with malnutrition or immunodeficiency. The prevalence of giardiasis is 2-7% in industrialized countries and 40% in developing countries. In studies to assess the frequency of intestinal parasitic infection in Egypt, *Giardia lamblia* was recorded in 34.3% of stool samples by IFAT in a report from Menoufia governorate<sup>[1]</sup>; and 37% of water samples from different districts in Alexandria city also by flow cytometry<sup>[2]</sup>. A more recent report from greater Cairo recorded 26% infectivity<sup>[3]</sup>.

Although giardiasis is usually self-limited, it can develop into chronic and life-threatening disease. Chronic giardiasis in early childhood has been significantly associated with malnutrition disorders<sup>[4]</sup>. In the United States, analysis data on all giardiasis outbreaks (reported to the Centers for Disease Control from 1971 to 2011) showed 242 outbreaks, affecting approximately 41000 persons of whom 74.8% resulted from waterborne transmission. Most of these waterborne outbreaks were associated with drinking water, followed by recreational water (18.2%)<sup>[5]</sup>.

Of note, *Giardia* cysts are known to be resistant to chlorine at concentrations typically applied for water treatment<sup>[6]</sup>. Disinfection using irradiation technology is of growing interest in the food and water industry being very effective against many organisms<sup>[7]</sup>. In addition, ionizing radiation has more advantages over deficiencies of chemical processes. Ionizing radiation showed ability for converting non-biodegradable substances to more readily degradable ones without leaving residues which is why it is called clean technology<sup>[8]</sup>.

There are no preparations for proper prophylaxis against giardiasis in spite of the numerous sources of infection with *G. lamblia*, and the preventive use of medications could not be recommended, except for highly endemic areas<sup>[9]</sup>. Current efforts are focused on finding new alternatives to chemical treatment, such as irradiation using UV rays<sup>[10]</sup> and cobalt 60<sup>[11]</sup>. The high energy rays of irradiation cause direct damage to the DNA of living organisms inducing cross-linkages and other changes that render an organism unable to grow or reproduce. Additional damage to DNA of organisms is indirectly produced by the interaction of rays with intercellular water molecules generating transient free radicals<sup>[12]</sup>.

Since the early nineties, cobalt-60, a type of gamma radiation, has been used on food to control infectivity of several parasites as *Paragonimus westermani*<sup>[13]</sup>, *Metagonimus yokogawai*<sup>[14]</sup>, *Eimeria tenella*<sup>[15]</sup>, *Toxoplasma gondii*<sup>[16]</sup>, *Angiostrongylus cantonensis*<sup>[17]</sup>, *Taenia solium*<sup>[18]</sup>, *Clonorchis sinensis*<sup>[19]</sup>, *Cryptosporidium parvum*<sup>[20]</sup>, *Ascaris lumbricoides*<sup>[21]</sup>, *Trypanosoma cruzi*<sup>[22]</sup> and *Echinococcus granulosus*<sup>[23]</sup>. UV irradiation usage for the disinfection of drinking water was first applied in 1910 in Marseille. After that, UV radiation gained interest and during the 1980s, it has been largely used in Europe for the disinfection of drinking water; in some cases, it has been used to replace chlorination<sup>[24]</sup>.

Many studies have been made to investigate the effect of UV irradiation on food items. It diminished *Salmonella* species as well as *Escherichia coli* on fruits and vegetables<sup>[25]</sup>. For blood transfusion, UV treatment can also be an effective blood disinfection method against *Trypanosoma cruzi*<sup>[26]</sup> and *Leishmania donovani*<sup>[27]</sup>. It can also be used against *Cryptosporidium parvum*<sup>[28]</sup> and soil nematodes<sup>[29]</sup>. For disinfections against contamination with *Toxoplasma gondii* cysts or oocysts one minute ultraviolet exposure inhibits tachyzoite replication and cyst conversion without diminishing host humoral-mediated immune response<sup>[30]</sup>. Concerning safety, a study carried by Kim *et al.*<sup>[31]</sup> assured the safe use of radiation and relieved the general anxiety about exposure to low-dose radiation. Moreover, Koca *et al.*<sup>[32]</sup> found that lack of acute adverse effects and low cost, seem to make radiotherapy one of the safest and cheapest treatment modality.

The present case control experimental study was conducted to evaluate the effect of radioactive cobalt-60 and 254 nm-UV irradiation on infectivity of *G. lamblia* cysts to mice, and examining their ability to reduce the cysts infectivity.

## MATERIALS AND METHODS

**Giardia cysts collection:** Stool samples were collected from outpatients and inpatients attending laboratories of Ain Shams and Cairo University hospitals, Cairo, Egypt, with bias to patients with symptoms suggesting giardiasis. Samples were collected in clean, dry and labeled plastic containers and were examined in the Parasitology Research and Diagnostic Laboratory Unit at Faculty of Medicine, Ain Shams University, Cairo, Egypt to detect positive cases for giardiasis.

**Purified Giardia cysts treatment:** Stored samples of *Giardia* cysts were concentrated and purified<sup>[33,34]</sup>, then divided into 3 equal parts for irradiation with cobalt-60, irradiation with UV, and the third part was kept without radiation. All samples were preserved at 4°C.

**Cobalt-60 irradiations:** Before irradiation, the sample was brought to room temperature in an open 55-mm glass

Petri dish. In the Medical Oncology Department, Ain Shams University, Cairo, Egypt, the sample was subjected to a direct field beam of radioactive cobalt-60 in a dose of 0.25 KGy, which gave an intensity of 1.25 MeV at a distance of 60 cm on an area of 20x20 cm with 0.5 bolus for 25 min<sup>[35]</sup>.

**Ultraviolet radiation:** The sample was put in a Petri dish (8.5 cm diameter) that was used as the static reaction vessel. The cysts were counted using a haemocytometer and diluted in physiological saline. The cyst preparations were assessed qualitatively for cyst aggregation by microscopy. The absorbance and percentage transmission of the suspension at 254 nm were measured at temperature 4.2°C, exposure time 120 seconds and 20 mJ cm<sup>2</sup><sup>[36]</sup>.

## Experimental infection of BALB/c mice with *G. lamblia* cysts:

Sixty male BALB/c mice, aged 4-8 weeks old and weighing 25-40 gm, were obtained from the animal house of the National Research Center, Cairo, Egypt and were caged in the medical research center animal house, 2-3 mice per cage<sup>[37]</sup>. The mice were proven free of giardiasis by microscopically examining their stool on three consecutive days. The animals were divided into 6 groups each composed of 10 mice: **group 1** infected with a suspension of human derived *Giardia* cysts irradiated with cobalt-60<sup>[35]</sup>; **group 2** infected with a suspension of human derived *Giardia* cysts irradiated with 254 nm-UV rays<sup>[38]</sup>; **group 3** infected with human derived non-treated *Giardia* cysts; **group 4** non-infected mice, received water irradiated with 0.25 KGy of cobalt-60; **group 5** non-infected mice, received water irradiated with 254 nm-UV rays; **group 6** non-infected mice, received non-treated water. The mice were inoculated orally by 0.2 ml suspension, for each mouse, containing  $1 \times 10^6$  cysts using an orogastric gavage, after overnight fasting<sup>[39]</sup>.

## Parameters for evaluation of the infectivity of *G. lamblia* cysts:

Fecal pellets were collected daily from each cage, starting from day 9 post inoculation<sup>[40]</sup> and examined microscopically. If cysts were detected, the animals were individually placed in separate cages and their stool pellets were re-examined. All stool pellets from individually housed mice were examined microscopically over a 24-h period till day 12.

All mice were sacrificed on day 21 post infection and duodenal aspiration was done. The aspirate was examined for *Giardia* cysts or trophozoites. Then, mice small intestines were opened by longitudinal slit<sup>[41]</sup> and parts of the small intestine were preserved in 10% formalin solution for further hematoxylin and eosin (H&E) staining and histopathological examination<sup>[42,43]</sup>.

## Histopathological examination of small intestine:

Small intestinal punch biopsy specimens were fixed, dehydrated and embedded in paraffin wax. Serial

sections of 5 micron thickness were stained with H&E. Histopathological evaluation was modified from Oberhuber and Stolte<sup>[44]</sup>. In all groups, sections were thoroughly examined to determine giardiasis, and confirmed by presence of sickle shaped trophozoites attached to surface epithelial cells or free within the mucus layer. The density of colonization was estimated semi-quantitatively (0; +: 1-10; ++: 10-30; +++: >30). Inflammation was assessed semi-quantitatively by estimating the quantity of inflammatory cells present in the lamina propria in random high power microscopic fields. Score 0 = comparable to uninfected control (group 6); score 1 = slightly increased intensity of inflammation; score 2 = moderate increase in intensity of inflammation; and score 3 = severe inflammation compared to score 2. The degree of villous flattening was assessed semi-quantitatively. Grade 0 represented normal villi; grade 1 was defined as mild villous flattening; grade II represented short blunted villi; and grade III represented subtotal villous flattening. The results were expressed as the number of intra epithelial lymphocytes for each 100 epithelial cells. Lymphoid follicles were defined as an aggregation of lymphocytes with or without germinal center<sup>[44]</sup>.

**Statistical analysis:** For data management and analysis, the collected data was revised, coded, tabulated and introduced to a PC using statistical package for Social Science (SPSS 15.0.1 for windows; SPSS Inc, Chicago, IL, 2001). Data were presented and suitable analysis was done according to the type of data obtained for each parameter. For descriptive statistics, frequency and percentage of non-numerical data was used. For analytical statistics, Fishers exact test was used to examine the relationship between two qualitative variables when the expected count was less than 5 in more than 20% of cells.

**Ethical consideration:** An informed consent was taken from all patients before taking stool samples. The study was approved by the Research Ethics Committee, Faculty of Medicine, Ain Shams University. Ethical guidelines for experimental animals were considered aiming to alleviate or minimize potential pain, suffering, or distress and enhance the welfare of the animals used.

## RESULTS

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### Direct stool examination (Table 1 and Figure 1):

In group 1, one mouse (10%) was infected on day 11 and

remained infected till day 21. In group 2, all mice (0%) were non-infected all through the 21 days. In group 3, eight mice (80%) were infected on day 10 and by day 21 all 10 mice (100%) were infected. In groups (4, 5 and 6), no mice (0%) were infected all through the 21 days. All non-infected mice that received irradiated or non-irradiated water were not infected.

**Duodenal aspiration (Table 2 and Figure 2):** On day 21, microscopic examination of duodenal aspirates of group 1 showed trophozoites in four mice (40%); in group 2 no trophozoites (0%) were detected; in group 3 trophozoites were detected in all mice (100%).

**Histopathology (Table 3, Figures 3 and 4):** Histopathological examination of mice intestines revealed no trophozoites in the non-infected experimental control groups 4, 5 and 6. Trophozoites were detected in all of group 3 mice (100%) infected with non-irradiated *Giardia* cysts. Irradiation with cobalt 60 (group 1) or UV rays (group 2) reduced the percentage of infection to 50% ( $P=0.003$ ) and 20% ( $P=0.001$ ) respectively (Table 3). The intensity of colonization decreased from (++++) in group 3 to (+++/+) in group 1 and to (+) in group 2 ( $P<0.001$ ). The grade of inflammation due to infection with non-irradiated *Giardia* cysts (group 3) was  $2.80\pm 0.42$ . Irradiation of *Giardia* cysts with Cobalt 60 in group 1 decreased inflammation ( $1.70\pm 0.67$ ,  $P=0.0004$ ). Irradiation of *Giardia* cysts with UV rays in group 2 did not significantly decrease intestinal inflammation ( $2.4\pm 0.52$ ,  $P=0.739$ ). The inflammatory response to Cobalt 60 was less intense than UV rays ( $P=0.0179$ ), and the grade of inflammation ranged from  $1.30\pm 0.15$  in group 4, to  $1.7\pm 0.67$  in group 1 ( $P=0.1449$ ). The grade of inflammation due to UV rays ranged from  $1.9\pm 0.74$  in group 5 to  $2.4\pm 0.52$  in group 2, ( $P=0.0962$ ). In the infected experimental groups 1, 2 and 3, the villus changes ranged from first degree mild flattening to second degree short blunted.

The percentage of villus change observed in group 3 infected with non-irradiated *Giardia* cysts significantly decreased when the cysts were irradiated with cobalt 60 in group 1 ( $P=0.033$ ). Irradiation with UV rays in group 2 did not significantly alter the villus changes. No difference in the villus architecture occurred when the cysts were irradiated with cobalt 60 in group 1 or UV rays in group 2 (Table 3).

**Table 1:** Results of direct stool examination for *Giardia* cysts for groups 1, 2 and 3 (21 days after infection)

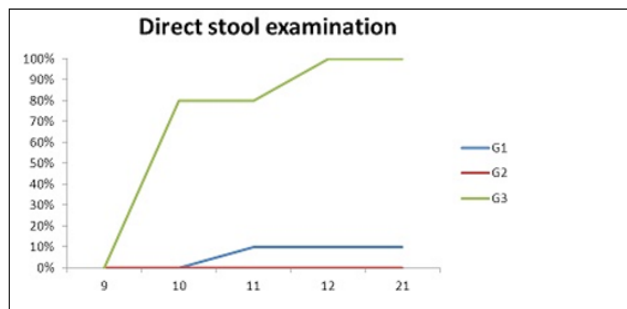
	Positive Number	%	Negative Number	%	Fisher exact test	
					P value	Significance
G1	1	10%	9	90%	1	NS
G2	0	0%	10	100%		
G1	1	10%	9	90%	<0.001	S
G3	10	100%	0	0%		
G2	0	0%	10	100%	<0.001	S
G3	10	100%	0	0%		

**Table 2:** Results of duodenal aspirate examination for *Giardia* cysts for groups 1, 2 and 3 (21 days after infection)

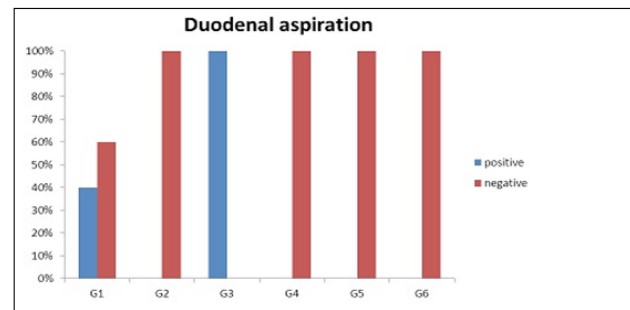
	Positive Number	%	Negative Number	%	Fisher exact test	
					P value	Significance
G1	4	40%	6	60%	0.087	NS
G2	0	0%	10	100%		
G1	4	40%	6	60%	<0.011	S
G3	10	100%	0	0%		
G2	0	0%	10	100%	<0.001	S
G3	10	100%	0	0%		

**Table 3:** Histopathological results of groups 1, 2 and 3 showing 2 parameters (trophozoite existence and villous changes)

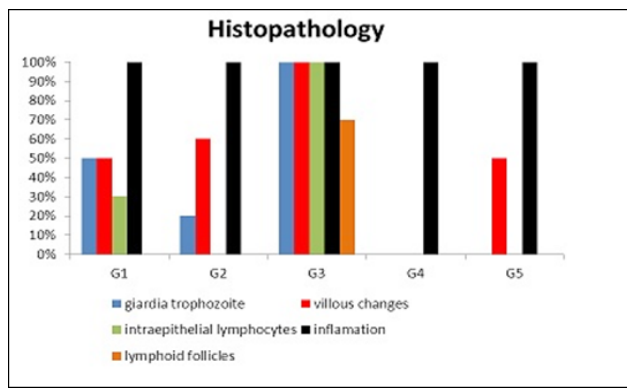
	<i>Giardia</i> trophozoite						Villus changes					
	Positive		Negative		Fisher exact test		Positive		Negative		Fisher exact test	
	N	%	N	%	P value	Sig.	N	%	N	%	P value	Sig.
G1	5	50%	5	50%	0.350	NS	5	50%	5	50%	1	NS
G2	2	20%	8	80%			6	60%	4	40%		
G1	5	50%	5	50%	0.033	S	5	50%	5	50%	0.033	S
G3	10	100%	0	0%			10	100%	0	0%		
G2	2	20%	8	80%	0.001	S	6	60%	4	40%	0.087	NS
G3	10	100%	0	0%			10	100%	0	0%		



**Fig. 1:** Percentages of infected mice using direct stool examination through days 9, 10, 11, 12 and 21.



**Fig. 2:** Percentages of infected mice using duodenal aspiration examination through days 9, 10, 11, 12 and 21.

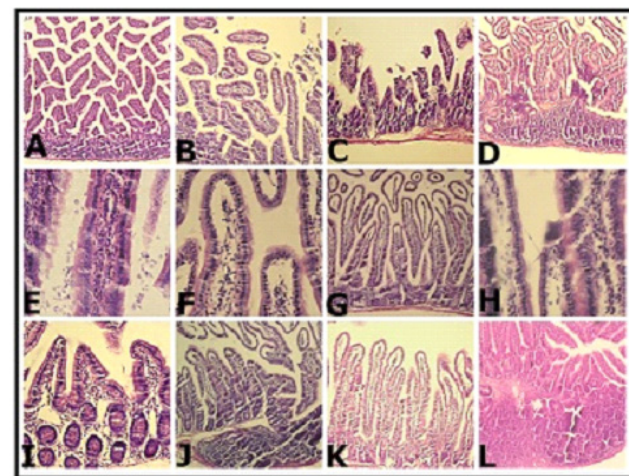


**Fig. 3:** Percentages of infected mice showing histopathological changes in the intestines of study groups

## DISCUSSION

The present study is an attempt to determine the efficacy of radioactive cobalt-60 and 254 nm-UV irradiation on infectivity of *G. lamblia* cysts to mice. Results showed that experimental irradiation of *Giardia* cysts with cobalt-60 at a dose of 0.25 KGy (group 1) decreased infectivity to 50% in comparison to 100% infectivity in non-irradiated group (group 3) as observed by histopathological examination. Lenaghan and Sundermann<sup>[45]</sup> studied the effect of variable doses of the radioactive cobalt-60 on the *Giardia* trophozoites and concluded that a dose of more than 5 KGy is sufficient to inactivate the trophozoites and render them unable to recover. Another study found that *Giardia* cysts exposed to a dose of 2.0, 1.0, 0.75, 0.5, 0.46, or 0.25 KGy were not infective to gerbils<sup>[46]</sup>. Also, El-Rifaey *et al.*<sup>[7]</sup> studied the effectiveness of radioactive cobalt-60 in the inactivation of *G. lamblia* cyst in prevention of infection and concluded that radioactive cobalt-60 at a dose of 0.25 KGy can be used as a control measure to prevent infectivity and as a method of water treatment.

Results of our study showed that UV irradiation using wave length 254 nm reduced the infectivity of cysts to 20%. This was determined by histopathological examination of mice small intestines on day 21 post-infection. In accordance, Campbell and Wallis<sup>[36]</sup> reported that 254 nm UV irradiation successfully inactivated *Giardia* cysts at 4°C after 21 days. Also, Mofidi *et al.*<sup>[47]</sup> recorded the same results using two different strains, *G. lamblia* and *G. muris*, with UV dosages (ranging from 0.4 to 9.8 mJ/cm<sup>2</sup>). Shin *et al.*<sup>[48]</sup> found that *G. lamblia* cysts were very sensitive to UV irradiation. One year later, the same investigators attempted reactivation of these UV exposed *Giardia* cysts and recorded no evidence of reactivation<sup>[49]</sup>. dos Santos *et al.*<sup>[50]</sup> studied the effect of infection of BALB/c mice by cysts exposed to UV radiation dose 25-30 mJ cm<sup>2</sup> and found that the mice released a lower concentration of cysts in their stool than those inoculated with non-irradiated cysts. Recently, it was proved that UV radiation at 10 mJ cm<sup>2</sup> can kill *Giardia* cysts effectively<sup>[51]</sup>.



**Fig. 4:** Histopathological examination of mice intestines. **Group 6:** Non-infected control group that received non-treated water shows normal intestine (A, H & E x100). **Group 3:** Infected with non-irradiated *Giardia* shows variable degrees of villus changes (B, C, H & E x100), inflammation (D, H & E x100) and intensity of colonization +++ (E, H & E x400). **Group 1:** Irradiation with Cobalt 60, decreased the intensity of colonization to ++ (F, H & E x400), decreased inflammation and preserved villi (G, H & E x100). **Group 2:** Irradiation with UV rays decreased the intensity of colonization to + (H, H & E x400), but UV rays didn't significantly decrease villus damage (I, H & E x100) or inflammation (J, H & E x100). **Group 4:** Non-infected control group received Cobalt 60. **Group 5:** Non-infected control group received UV; Cobalt 60 and UV didn't significantly damage the intestinal villi (K, L), however, Cobalt 60 (K, H & E x100) elicited lower inflammatory response than UV rays (L, H & E x100).

Histopathological findings in our study demonstrated that radioactive cobalt-60 and 254 nm-UV irradiation treatments are effective disinfection methods against *G. lamblia*. Both of them efficiently reduced the infectivity of *G. lamblia* cysts. UV irradiation treatment proved to be a more powerful disinfection method than cobalt-60, having controlled the rate of infection and intensity of colonization more than the latter. However, cobalt-60 modified the virulence of the organism while preserving the mucosal architecture in the infected mice. The rays of UV, to some extent, degraded the intestinal villi more frequently than cobalt-60. This difference was statistically insignificant. Although both techniques apparently did not damage the intestinal villi, the UV irradiated cysts elicited a higher inflammatory response than the cobalt-60 irradiated ones. These considerations should be borne in mind when choosing the method of disinfection pending that safety issues and cost-effectiveness allow it.

In conclusion, this study demonstrated that low dose radioactive cobalt-60 and UV irradiation at 254 nm may be considered as a mean of water treatment and to prevent *G. lamblia* infection. Further studies are recommended to assess the effectiveness of water irradiation using both UV and cobalt-60 gamma rays together. Smaller doses of each may be tested to gain advantages of UV in irreversible damage of *Giardia* DNA and the less inflammatory changes associated with cobalt-60.

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