Experimental study on the use of Cobalt-60 irradiation in the inactivation of Giardia Lamblia cysts

Thesis

Submitted for fulfillment of M.Sc. Degree in **Parasitology**

By

Rita Maher Kelada (M.B.B.Ch.)

Resident of Medical Parasitology

National Hepatology and Tropical Medicine Research Institute

(NHTMRI)

Under the Supervision of

Prof. Dr. SOAD ABDEL SALAM ELRIFAEY

Professor of Parasitology

Cairo University

Dr. AMIRA HASSAN ELNAMAKY

Assistant professor of Parasitology and Animal Diseases

National Research Centre

Dr. EMAN YASSIEN SHOEIB

Lecturer of Parasitology

Cairo University

FACULTY OF MEDICINE

CAIRO UNIVERSITY

2012

Acknowledgment

*First, before all, thanks be to **GOD** this work has been accomplished.*

*I would like to express my deepest gratitude to **Dr. Soad El Rifaey**, Professor of Medical Parasitology, Cairo University, for her care, continuous encouragement and guidance.*

*I wish to offer my sincere thanks to **Dr. Amira ElNamaky**, assistant professor of Parasitology and animal disease, National Research Centre , for her valuable assistance and support, which have always been beyond description.*

*I wish to offer my sincere thanks to **Dr. Eman Yassien**, lecturer of Medical Parasitology, Cairo University, for her valuable assistance and support, which have always been beyond description.*

*I could not forget the great help of **Dr. Raafat Abdel Malek**, Lecturer of Clinical Oncology, Cairo University and Director of NEMROCK clinical research unit, during the accomplishment of this work.*

*I also could not forget the great help of **Dr. Ehsan Hassan,** head of pathology department, National Hepatology and Research Institute, Cairo, during the accomplishment of this work.*

My deepest thanks are sincerely offered to all my professors and colleagues for their encouraging attitude.

Abstract

Giardia lamblia is a major cause of diarrheal disease all over the world. It is transmitted via the oral route in the cyst stage which is very resistant to disinfectants commonly used in drinking-water treatment.

The present work was designed to assess the ability of radioactive Cobalt to inactivate ***G. lamblia*** cysts thus they would not be infective to BALB/c mice.

One hundred and twenty mice were used and divided into four groups. The first group included 50 mice, infected with a suspension containing human derived non-irradiated ***G. lamblia*** cysts and served as control. The second group included 50 mice, which was the experimental group and was infected with a suspension containing human derived ***G. lamblia*** cysts that received a dose of 0.25Kilogray of Cobalt-60. The third group (10 mice) received irradiated water with a dose of 0.25 KGy of Cobalt-60 and the fourth group didn't receive anything. The infectivity was assessed by stool examination and the presence of trophozoites in the small intestine of inoculated mice. Stool examination was done using three different techniques: simple sedimentation, the Mini Parasep SF® and the rapid Copro-Antigen detection kit. Moreover, histopathological examination of the mice' small intestine revealed presence of trophozoites in group A but not in group B.

The cysts that received the radioactive Cobalt were unable to induce infection in the inoculated mice; while the infectivity of the non-irradiated cysts was 95.5%. Among the three methods used in the diagnosis the most sensitive was the copro-antigen with sensitivity of 95.5 % followed by the parasep with sensitivity of 86.6% and lastly the simple sedimentation with sensitivity of 75.5%.

The results of the present study indicate that radioactive Cobalt is very effective against ***G. lamblia*** cysts and can be used as a control measure to prevent infectivity.

Keywords: ***G. lamblia – Cobalt-60 – Parasep – Copro-Antigen***

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List of abbreviations

Abbreviation	Full name
Co-60	Cobalt 60
DFA	Direct Fluorescent Antibody
DNA	Deoxyribo Nucleic Acid
ELISA	Enzyme Linked Immunosorbent Assay
FDA	Food and Drug Administration
G.lamblia	Giardia lamblia
KGy	Kilogray
NASA	National Aeronautics and Space Administration
O_3	Ozone
PET	Positron Emission Tomography
USDA	United States Departement of agriculture
UV	Ultraviolet

Introduction

Water pollution is a major problem in the global context. It has been suggested that it is the leading worldwide cause of deaths and diseases **(Pink, 2006).** The specific contaminants leading to pollution in water include a wide spectrum of chemicals, pathogens and physical changes. Pathogens can produce waterborne diseases in either human or animal hosts. **(Hogan and McGinley, 2010)**

Recently there has been a sharp increase in the number of waterborne outbreaks caused by ingestion of some intestinal protozoa (**De Regnier *et al*., 1989 and Tibbets, 2000).**

Giardiasis has been one of the most frequently occurring waterborne diseases in both developed and developing countries. **(Meyer, 1980)**

The etiological agent, *Giardia lamblia*, is an intestinal parasite transmitted via the oral route in the cyst stage which is known to be very persistent in water and extremely resistant to the disinfectants commonly used in drinking-water treatment. **(Black *et al*., 1977 and Pickering & Engelkirk, 1990)**

Outbreaks of giardiasis have been most common in small water supply systems that often use chlorination only for treatment **(Craun *et al*., 1986 and Kent *et al.*, 1988)**. A need for low-maintenance, cost-effective treatment for these systems has been recognized, and alternative technologies are being investigated.
Different types of radiation as ultraviolet radiation, ozone and gamma rays are being considered as potential alternatives to chlorine for

disinfection in small water systems. **(Labatiuk *et al.*, 1991 and Rice and Hoff, 1981)**

Since the early nineties Cobalt-60، a type of gamma radiation، has been used to control infectivity of several parasites as *Clonorchis sinensis* and *Toxoplasma gondii* **(Song *et al.*, 1993 and Song *et al.*, 1994)**; however, only few studies have tested its effect on *Giardia lamblia* cysts (**Sundermann and Estridge**, **2010**).

Aim of work:

This is an experimental study aiming to evaluate the effect of radioactive Cobalt-60 radiation on the viability and infectivity of *Giardia lamblia* cysts for possible further use in disinfection purposes.

Review of literature

Giardia lamblia (syn. *Giardia intestinalis, Giardia duodenalis*) is a flagellated unicellular eukaryotic microorganism that commonly causes diarrheal disease throughout the world. It is the most common intestinal protozoan parasite, causing diarrhea in children, especially those with malnutrition or with immunodeficiencies **(Campbell, 1992 and Hakim *et al*., 2011).** In certain areas of the world, water contaminated with *G. lamblia* cysts commonly causes travel-related *Giardia*sis in tourists **(Versy and Peterson,1999).**

Taxonomy **(Ortega and Adam, 1997)**

Domain:	Eukaryota
Phylum:	Metamonada
Order:	Diplomonadida
Family:	Hexamitidae
Genus:	*Giardia*
Species:	*G. lamblia*

The taxonomy and host specificity of *Giardia* remain the subject of considerable debate. The organism has been found in more than 40 animal species **(Sulaiman *et al.*, 2004)**. Nowadays, five species of *Giardia* are established in the scientific literature, including the three species proposed by **Filice** in **1952**: *G. muris* in rodents, birds, and reptiles, *G. intestinalis* (syn: *duodenalis*, syn: *lamblia)* in mammals (including man), rodents, reptiles, and possibly birds, *G. agilis* in amphibians; *G. psittaci* in the budgerigar **(Erlandsen and Bemrick, 1987)** and *G. ardae* in the great blue heron (**Erlandsen *et al*., 1990).** A morphologically distinct *Giardia* isolated from the straw-necked ibis was later suggested to be a strain of *G. ardae.* **(Sulaiman *et al*., 2004)**

History of the discovery and species designation of *Giardia*:

Giardia was initially described by Van Leeuwenhoek in 1681 as he was examining his own diarrheal stools under the microscope **(Strickland, 2000).** The organism was described in greater detail by Lambl in 1859, who thought the organism belonged to the genus *Cercomonas* and named it *Cercomonas intestinalis* **(Strickland, 2000).** Thereafter, some have named the genus after him while others have named the species of the human form after him (i.e., *G. lamblia*). In 1879, Grassi named a rodent organism now known to be a *Giardia* species, *Dimorphus muris*, apparently unaware of Lambl's earlier description. In 1882 and 1883, Kunstler described an organism in tadpoles (?*G. agilis*) that he named *Giardia*, the first time *Giardia* was used as a genus name. In **1952**, **Filice *et al.*** published a detailed morphologic description of *Giardia* and proposed that three species names be used on the basis of the morphology of the median body: *G. duodenalis, G. muris*, and *G. agilis* . The species name *G. lamblia* became widely accepted through the 1970s. Since the 1980s, some have encouraged the use of the name *G. duodenalis*, and in the 1990s, the name *G. intestinalis* has been encouraged by other investigators (**Kulda**, **1998**). At that time the term *G. lamblia* has been widely accepted in the medical and scientific literature.

A number of molecular genetic techniques have been employed successfully to characterize *Giardia* isolates**.** In the case of G. *intestinalis*, molecular studies have demonstrated at least eight major genotypes or assemblages (**Thompson and Monis**, **2004**), some of which appear to have restricted host ranges (**Monis *et al***., **2003**). Only two of these Assemblages, A and B, have genotypes that have been isolated from humans (**Thompson and Monis**, **2004**). Nucleotide sequence of the triosephosphate isomerase (TPI) has been used to genotype G. *intestinalis* (**Sulaiman *et al***., **2004**) because of the high genetic heterogeneity displayed by *Giardia* spp. at this locus (**Monis *et al*., 1999). Sulaiman *et al*. (2004)** confirmed, basing on TPI sequences, that some animal isolates of G. *intestinalis* are of zoonotic potential, and they suggest that TPI should be useful in the detection, differentiation, and taxonomy of *Giardia* spp.

Epidemiology

Giardia lamblia is worldwide in distribution and is especially common in areas with poor sanitary conditions and insufficient water treatment facilities prevail **(Ortega and Adam, 1997)**. *Giardia*sis is also found among people living in developed countries where sanitation is adequate and water supplies are piped and purified (**Mank, 2001**). Worldwide the highest prevalence of *Giardia*sis occurs in the tropics and subtropics **(Strickland, 2000).**

The prevalence of *Giardia* in stool specimens is 2% - 7% in industrialized countries as USA and 40 % in developing countries. **(Hakim *et al*., 2011)**

In Egypt, there are previous epidemiological data about *G. intestinalis* in humans. **Zaki *et al. (*1986**) found *G. intestinalis* in 44% of the population studied in rural zones and **Shukry *et al.* (1986)** in 33% of the people in Cairo and **Curtale *et al*. (1998)** studied faecal samples and detected G. *intestinalis* in

24.7% of the samples in Behera Governorate. **Mahmud *et al*. (2001)** found antibodies against *G. lamblia* in samples from infants in Bilbeis.

There are several works about people with diarrhea; in this case, **Fawzi *et al*. (2004**) detected G. *intestinalis* in 10.4% of the studied fecal samples in Alexandria. **El-Shazly *et al*. (2004)** detected genotypes I and II that belong to Assemblage A, as well as Genotype III that belongs to Assemblage B, in humans. . **El Shazly *et al***. (2006) reported the presence of G.*lamblia* in 19.6% of all patients attending Mansoura Hospital in Dakahlia Governorate.

A recent study conducted in Egypt revealed that the highest prevalence of G. *intestinalis* was found in Gharbia, with 44.4% of the faeces positive, followed by Ameria 38.5%, and Kafr El Sheik 25%. In the last two places, 100% of the positive samples were Assemblage B. **(Foronda *et al*., 2008)**

In a recent study conducted in Minia district, **Abdel Hafeez *et al*. (2012)** found prevalence of G.*lamblia* among children to be 17.6% and reported that *Giardia* together with *Entamoeba histolytica* are more common than other protozoa in immunocompetent children On the other hand, **Abdel Salam** (**2012**) found *G. lamblia* in 10% of swimming pool samples in Alexandria.

In developing countries in Asia, Africa and Latin America, approximately 200 million people have symptomatic *Giardia*sis (**Thompson *et al*., 2000 and Yason and Rivera, 2007**).

In Africa, the percentage of people expelling *Giardia* cysts was reported to be 17.3% in South Africa (**Adams *et al*., 2005**), 11.7% in Morocco **(El Kettani *et al*., 2006),** 2- 11.4% in Ethiopia **(Gelanew *et al*., 2007),** 29% in Sierra Leone **(Gbakima et al., 2007)** and 13.9% in Cote d'Ivoire (**Quihui *et al.*, 2010**).

*Giardia*sis has an estimated worldwide prevalence of 280 million cases annually. Furthermore it contributes substantially to the 2.5 million annual deaths from diarrheal disease **(Strickland, 2000).**

Abreu-Acosta *et al. (*2007*)* have detected differences between the prevalence of protozoa when they used morphological and molecular methods, obtaining higher prevalence with the molecular one. Therefore, prevalence data based on morphological detection most certainly underestimate true prevalence because of its low sensibility (**Abreu-Acosta *et al*., 2007).**

Morphology and life cycle

The trophozoite form **(fig. 1a)** is tear drop shaped with a pointed posterior end, measuring 10 to 20 μm in length and 5 to 15 μm in width, laterally resembles the curved portion of a spoon, the concave portion is the area of the sucking disc. There are four pairs of flagella, two nuclei, two axonemes and two slightly curved bodies called the median bodies **(Garcia, 1997).** The cytoskeleton of trophozoites, in particular the ventral disc, is believed to play a major role in its attachment to the host intestine **(Adam, 2001).** Proteins that are found exclusively on the ventral disc, such as actinin, alpha-actinin, myosin and tropomysin **(Feely *et al*., 1982),** as well as lectin **(Farthing *et al*., 1996),** have been proposed as biochemical agents involved in attachment. The four pairs of flagella are located on the anterior, posterior, caudal and ventral side of the organism. Each is composed of 11 microtubules. **(Corrêa *et al*., 2004).**

The cyst form **(fig. 1b)** may appear round or oval, measuring 11 to 14 µm in length and 7 to 10 µm in width. They contain four nuclei at one pole, axonemes, and median bodies surrounded by a cyst wall **(Garcia, 1997).** The cyst is covered by a 0.3~0.5um-thick cyst wall. The cyst wall is composed of two layers: outer filamentous layer and an inner membranous layer (further composed with two membranes). One cyst matures into two trophozoites. (**Adam**, **2001**)

Both the trophozoite and the cystic stages are involved in the life cycle **(fig. 2).** On ingestion of cysts they reach the upper part of small intestine, where each cyst liberates 2 trophozoites. They are most commonly detected in the crypts within the duodenum. Trophozoites may remain attached or detached from the mucosal surface. Cyst formation takes place as the organism move down through the colon. Both forms (cyst and trophozoite) are excreted in the stool. **(Garcia, 1997 and Pickering, 2000)**