e - ISSN - 2394-7721

American Journal of Oral Medicine and Radiology

Journal homepage: www.mcmed.us/journal/ajomr

THE VIABILITY TESTING OF *GIARDIA LAMBLIA* CYST

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Article Info

Received 16/10/2014
Revised 23/10/2014
Accepted 05/11/2014

Key words:- *Giardia lamblia*, Viability.

ABSTRACT

The parasite *Giardia lamblia* is a very simple eukaryote that is a unicellular protozoan parasite causes giardiasis, which is prevalent throughout the world. Patients complain of several symptoms as gastroenteritis that manifests itself with severe abdominal pain and diarrhea .This study was designed to determine the impact of different environmental conditions on the viability of *Giardia lamblia* cysts. One gram of faeces was dissolved in 35ml of Normal Saline. *Giardia lamblia* cysts were counted by using Haemocytometer and were exposed to different temperature as follows 100, 70, 60, 50, 40, 25, 15, 4, -20, Each of them was left for three different incubation periods as follows 15min,3 0min, 60min, Viability testing was done for the samples by using super-vital stains. Result the study found that the cysts survived for 15min when exposed to temperatures 70^{o}C, 60^{o}C, 50^{o}C, 40^{o}C, 37^{o}C, 25^{o}C, 15^{o}C, 4^{o}C, and -20^{o}C. While they survived for 30min is when exposed to temperatures 60^{o}C to 20-^{o}C. Furthermore they were survived for 60min when exposed to temperatures from 60^{o}C to 4^{o}C. This study concludes that *Giardia lamblia* cyst cane survive longer time at temperatures between 60 and 4^{o}C and cannot resist 100^{o}C.

INTRODUCTION

Giardia lamblia (synonymous with *Giardia intestinalis*, *Lamblia intestinalis* and *Giardia duodenalis*) is a flagellated protozoan parasite that colonizes and reproduces in the small intestine, causing giardiasis The parasite attaches to the epithelium by a ventral adhesive disc, and reproduces via binary fission. The parasite has two stages in its life cycle, the cyst and trophozoite. Infections are one of the most frequent waterborne causes of diarrhea worldwide. But more prevalent in third world countries where sanitation is bad and hygienic standards are low .the parasite passé from person to person by fecal mouth mechanism or by ingesting contaminated food or water [1]. Giardiasis a type of gastro enteritis that manifested itself with severe diarrhea and abdominal cramps. Giardiasis does not spread via the bloodstream, nor does it spread to other parts of the gastrointestinal tract, but remains confined to the lumen of the small intestine [2]. *Giardia* trophozoites absorb their nutrients from the lumen of the small intestine, and are anaerobes. If the organism is split and stained, its characteristic pattern resembles the familiar "smiley face" symbol.

Viability test of the cyst stag of the parasite done by several methods some of them are the staining by1% Giemsa stain, iodine and methylene blue and lacto phenol blue or by in vitro excystation [3].

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MATERIAL AND METHODS

Study type and design

This was an experimental study. In which the viability of *Giardia lamblia* cyst was evaluated following treatment with several degrees of temperature with different time durations.

Study variables

Results obtained by microscopic examination of stool sample in relation to different staining methods, different temperatures and different time of exposure.

Method and tools

Sample collection and storage

Sample was collected from a patient infected with giardiasis attending Omdurman teaching hospital.

Assay procedure

***Giardia lamblia* cyst purification**

One gram of feces was dissolved in 35 ml of normal saline for purification of the parasite. The sample was strived with tea striver to remove the large parasite there after the sample was transferred to centrifuge tube and centrifuge at 1000 rpm speed for one minute to concentrate the parasite.

Parasite count

Gardia lamblia cysts were collected by using haemocytometer on WBCs squires and multiplied by 50. The mean count was 3100 cyst\ml therefore the purified sample was divided in to 10 test tube 1ml \each. Cyst count was checked for each aliquot to insure that count is still 3100 as the stock sample. Each tube was exposed to different temperature as follows 100^{o}C ,70^{o}C ,60^{o}C,50^{o}C, 40^{o}C ,37^{o}C 25^{o}C ,15^{o}C, 4^{o}C, 20-^{o}C.each of them was left for three different incubation as follows 15min 30min 60min.

Viability testing

Using 1%Geimsa stain

A 50 ml of 1% Giemsa stain (appendix-3) was added to 1ml of each tube. The sample was transferred to haemocytometer. The viable cysts (none stained) were counted. The non-viable cysts stained with violet color.

Using 1% Methylene blue

A50 ml of 1% Methylene blue stain was added to 1ml of each tube. The sample was transferred to haemocytometer. The viable cysts (none stained) were counted. The non-viable cysts stained with blue color.

Using Iodine stain

A50 ml of Iodine stain was added to 1ml of each tube. The sample was transferred to haemocytometer. The viable cysts (none stained) were counted. The non-viable cysts stained with yellow color.

RESULTS

Fecal samples were collected and purified. *G. lamblia* cysts counts were 3100 cyst/ml and exposed to different grades of temperature (-20, 4, 15, 25, 37, 40, 50, 60, 70, 100^{O}C) for 15, 30 and 60 minutes.

After exposing the sample to the above mentioned degrees of temperature for 15min, the highest viability rate was recorded for cysts exposed to 37 ^{O}C and 25 ^{O}C. While the lower viability rate was recorded for cysts exposed to -20 ^{O}C.

Furthermore, cysts exposed to 100 ^{O}C were not viable (viability rate is zero) as shown in table 1.

While after exposing for 30 minutes, the highest viability rats were recorded for cysts exposed to 37 ^{O}C and 25 ^{O}C. While the lower viability rates were recorded for cysts exposed to -20 ^{O}C Furthermore cysts exposed to 100 ^{O}C and70 ^{O}C were not viable (viability rate is zero) as shown in table 2.

When we exposed for 60 minutes, the highest viability rate were recorded for cysts exposed to 37 ^{O}C and 25 ^{O}C. While the lower viability rates were recorded for cysts exposed to -20 ^{O}C. Furthermore cysts exposed to 100^{O}C and70 ^{O}C were all dead (viability rate is zero) (table 2).

- Total cyst before the sample strived 2650
- Total cyst after the sample strived 2000
- Total cyst after the sample centrifugation 3100

Table 1. The Viability % of *Giardia lamblia* cysts after exposure to different degrees of temperature for 15 min.

Temperature ^{o}C	Total cyst/ml	Viable cyst/ml	Viability %
100	3100	zero	zero
70	3100	2800	90 %
60	3100	2500	81 %
50	3100	2550	82 %
40	3100	3000	97 %
37	3100	3100	100 %
25	3100	3100	100 %
15	3100	2800	90 %
4	3100	2600	84 %
- 20	3100	1000	32 %

Table 2. The Viability % of *Giardia lamblia* cysts after exposure to different degrees of temperature for 30 min.

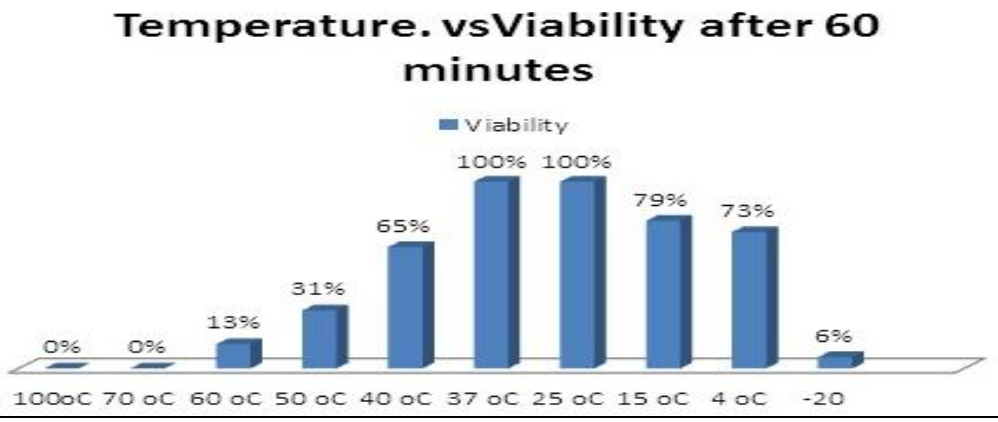
Temperature ^{o}C	Total cyst/ml	Viable cyst/ml	Viability %
100	3100	zero	zero
70	3100	zero	zero
60	3100	2000	65 %
50	3100	2200	71 %
40	3100	2900	94 %
37	3100	3100	100 %

25	3100	3100	100 %
15	3100	2800	90 %
4	3100	2600	84 %
-20	3100	600	26 %

Table 3. The Viability % of *Giardia lamblia* cysts after exposure to different degrees of temperature for 60 min.

Temperature ^{o}C	Total cyst/ml	Viable cyst/ml	Viability %
100	3100	zero	zero
70	3100	zero	zero
60	3100	400	13 %
50	3100	950	31 %
40	3100	2000	65 %
37	3100	3100	100 %
25	3100	3100	100 %
15	3100	2450	79 %
4	3100	2250	73 %
-20	3100	200	6 %

Figure 1. Effects of different temperatures on *Giardia lamblia* cysts



DISCUSSION AND CONCLUSION

In the present study the effect of temperature on *Giardia lamblia* cyst viability was tested under a variety of conditions. The experimental variables employed included temperature (100, 70, 60, 50, 40, 37, 25, 15, 4, and -20), and incubation time (15, 30, and 60 minutes), because of water temperature coupled with incubation time proved to be important in cyst survival. In our study it is interesting to note that according to our results the highest viability rate was recorded for cysts exposed to 37 ^{O}C and 25 ^{O}C. While the lower viability rate was recorded for cysts exposed to -20 ^{O}C. Furthermore, cysts exposed to 100 ^{O}C were not viable (viability rate is zero), this is in agreement with which report that, the biocidal effect caused by sunlight is due to the thermal processes that occur at temperatures above 45^{o}C.When exposing *G. lamblia* cysts to above mentioned temperatures for 30 mints also there was highest viability recorded in 25^{o}C and 37^{o}C, but there were no viability detected in 70 and100 this is more or less could explain that the majority of cysts will die when exposing to long duration, the same result was reported in study conducted at University of Zimbabwe who found that Both solar radiation and heat produced by the sun have a synergistic effect in killing cysts of *Giardia duodenalis* when temperatures rise above 50 degrees C, with complete death at 56 degrees. Also study conducted in Egypt they conclude that when put two vial of samples were exposed to sun in 2 exposures (6 & 24 hrs), high parasites death was recorded when tubes exposure to sun for 24 hrs [5-7]. In study conducted in United States of America reported that *G. lamblia* cysts survived equally well in 21 ^{o}C up to 60 min. these findings agreed with our result that cysts were survived for 60 min after were exposed to 25 ^{o}C. Hence the slightly high and low temperature will some bacterial cells, also takes place for particular parasites under study. Our study concludes that *G. lamblia* cyst can survive a longer time at temperatures between 60 ^{o}C and 4 ^{o}C and cannot resist at 100 ^{o}C. This is explaining that why *Giardia lamblia* cyst was more frequent parasites in our country.

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