*Available online at* www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

**Der Pharma Chemica, 2016, 8(10):99-101**
*(http://derpharmachemica.com/archive.html)*

# Role of Speed and Time of Centrifugation in Death of *Trichomonas galina* Trophozoites

**Ebrahim Badparva, Hossein Mahmoudvand and Sajad Padparva***

*Department of Medical Parasitology and Mycology, Lorestan University of Medical Sciences, Khorramabad, Iran*

---

**ABSTRACT**

*Trichomonas galina is a protozoan parasite that lives in digestive system of birds in form of trophozoite. Regarding the urgent need of most of the diagnostic methods for applied centrifugal devices, we were prompted to examine, for the first time, this parasite's tolerance against variables of speed (RPM: revolution per minute) and time and, meanwhile, take into account the subtlety of the techniques by increasing the sensitivity of the methods. At first, 0.5ml (approximately $5\times10^5$ parasites) of the culture medium (growth medium) was added to each of the two series of 14-piece microtubes containing 1.5ml of reinforced RPMI 1640 culture medium. Then, after mixing, except microtube (1) kept as the standard, the first series was centrifuged at speed of 1 to13000 rpm and the second series was centrifuged for 1 to 13 minutes; next, both series, after 24 hours of incubation, were counted and calculated, compared to the standard. T. galina well tolerated up to the speed of 6000rpm, then it tolerated up to 9000rpm with a significant gradual decline; however, it was completely vanished at speed of 10000rpm and above. While, the second series well tolerated up to the ninth minute of the centrifugation but, from then onward, it demonstrated a gradual declination of tolerance. Only 40% of the parasites survived until the last minute of the centrifugation time, i.e. the thirteenth minute. It was found that the threshold tolerance of centrifugation speed for these parasites is 10000rpm and the effect of increasing the centrifugation speed on the parasite's death is much more than that of increasing the centrifugation time. Although, the result of this study is specific to T. galina and cannot be generalized due to specific biological characteristics of each parasite, it can be a starting point for future investigations on the details of methods so that, as long as the device settings are concerned, references should be carefully cited.*

**Keywords:** *Trichomonas galina*, centrifugation speed, centrifugation time, death

---

## INTRODUCTION

*Trichomonas galina* is a flagellar protozoan parasite living in the digestive system of birds in form of trophozoite which is transferred directly or through water and food, breast feeding, or infected birds hunted by predator birds [1, 2]. This parasite, whose existence in birds has been proven in previous studies [3], causes the young birds' death and its consequent economic losses; however, the adult birds are carriers of this parasite [4]. The science of parasitology is based on ten indices including: name, geographical distribution, morphology, host, embedment place, evolution procedure, pathogenesis, diagnosis, treatment, and prevention [5]. Since programs for effective treatment, control, and prevention of parasitic diseases require fast, reliable, and highly sensitive diagnostic tests [6], the diagnosis can be considered as a key index.

There are various and numerous methods used for diagnosis of the parasites which can be divided into six categories: direct, stained, condensed, serological, culture, and molecular. Although sensitivity and characteristic as two assessment criteria [measure] are different for each method and each parasite [6-10], the majority of them have

in common the use of a machine called centrifuge with variables such as speed and time which are widely used in diagnosis, especially in condensation and precipitation of parasites.
The present study was aimed to investigate the effects of time and speed of centrifugation, as two important forgotten indices, on *T. galina* protozoan parasite in order to provide a starting point and pave the ground for taking into account the subtlety of the techniques, including the variables of the centrifugation device, along with increasing the sensitivity of the diagnostic tests.

## MATERIALS AND METHODS

In all laboratory phases, to prevent environmental disturbing factors, all devices and solutions (incubator, centrifuge, solutions such as culture medium and serum physiology, and even stool specimen) were kept at temperature of 38$^{o}$C, similar to the conditions within the body of the birds.

The suspension resulted from mixing the positive or parasite-containing samples, following direct diagnosis and in order to eliminate the disturbing matters, was passed through filters with pore diameter of 4mm$^2$ and centrifuged at speed of 4000rpm/5min. The resulting sediment, after discarding the supernatant, was cultured for 24 hours at temperature of 38$^{o}$C in 10ml of RPMI1640 culture medium reinforced with 10% inactive horse serum and about 6mg of rice starch without adding antibiotics in order for the parasite to grow and reach to the number of 1×10$^6$ml.

After making sure of reaching to the minimum required level of parasite growth in the second phase, 0.5ml (500000/ml parasites) was added to each of the two series of the two-millimeter 14-piece microtubes containing 1.5ml of the RPMI1640 culture medium and, after mixing, one of the microtubes was selected as the standard non-centrifuged microtube and the other 13 microtubes were centrifuged, based on their numbering, at speed of 1-13000rpm and, after remixing, were incubated for 24 hours at temperature of 38◦C. Next, in order to investigate the effect of the centrifugation speed, the results were counted using a Neubauer slide and the percentage of the living parasites was calculated in comparison with the standard per cubic millimeter (Table-I).

The fourth phase or investigation of the effect of centrifugation time on the parasite's growth was similar to the third phase except that, instead of the centrifugation speed, the centrifugation time was changed from1 to13 minutes. The results were again calculated as comparison with the standard (Table-II)

## RESULTS

The results of this study are shown as percentage of the living parasites compared to the standard or non-centrifuged microtubes in Table 1, which shows the effect of centrifugation speed, and in Table-II, which shows the effect of centrifugation time after 24 hours of culturing at temperature of 38◦C temperature counting by a Neubauer slide.

**Table 1. Effect of centrifugation speed on *Trichomonas* parasite's death in a constant time of 5 minutes**

| rpm ×1000 | Standard | 1 | 22 | 3 | 44 | 55 | 66 | 77 | 88 | 19 | 110 | 111 | 112 | 113 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Number of parasites per mm$^2$** | 36 | 36 | 34 | 35 | 34 | 33 | 34 | 13 | 12 | 5 | 0 | 0 | 0 | 0 |
| **Percentage of living parasites at any phase of speed** | 100 | 100 | 94.5 | 97 | 94.5 | 91.5 | 94.5 | 36 | 33 | 14 | 0 | 0 | 0 | 0 |

Up to the 6$^{th}$ tube, or at speed of 6000rpm, no significant difference with the standard p>% 5 was observed despite minor ups and downs; however, form the 7$^{th}$ tube onward, or at speed of 7000rpm, they affected the parasite's death, so that up to the 9$^{th}$ tube, by gradually increasing in the centrifugation speed, the number of the living parasites reached to 36%, 33%, and 14% , respectively. Further, at speed of 10000rpm onward, no living, moving, or active parasite was observed; in other words, the parasite couldn't tolerate the speeds of more than 10000rpm.

**Table 2. Effect of centrifugation time on *Trichomonas galina* parasite's death at constant speed of 6000rpm**

| Time in minute | Standard | 1 | 22 | 3 | 44 | 55 | 66 | 77 | 88 | 19 | 110 | 111 | 112 | 113 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Number of parasites per mm$^2$** | 37 | 37 | 37 | 39 | 40 | 37 | 35 | 35 | 34 | 35 | 30 | 22 | 18 | 15 |
| **Percentage of living parasites at any phase of time** | 100 | 100 | 100 | 100 | 100 | 100 | 94.5 | 94.5 | 92 | 94.5 | 81 | 59.5 | 48.5 | 40.5 |

As shown in Table 1, despite the ups and downs relative to the standard, the *T. galina* could well tolerate up to 9 minutes of centrifugation. The cases in which the number of the parasites was more than the standard in some phases due to the visual or technical errors were ignored. However, from the 10$^{th}$ minute onward, the decreasing trend began and reached its peak in the thirteenth minute but it never came to an end; that is, about 40% of the parasites were still alive at the end of the time.

## DISCUSSION

In this study, it was shown that the effect of the centrifugation speed is more effective on the death of *T. galina* parasite than that of the centrifugation time or, in other words, the parasite's sensitivity to centrifugation speed is more than to the centrifuging time, because they well tolerated the centrifugation time without any significant difference with the standard microtube and even 40% of them remained alive despite the gradual decrease up to the thirteenth minute of centrifugation despite a gradual decrease; while, up to the speed of 6000rpm, there was no significant difference with the standard microtube ( p>% 5) and since then decreasing trend was gradually increased and at the speed of 6000rpm no parasite was seen as living, active, or moving. In other words, all the parasites vanished at this phase.

Since no similar study on this field is reported, it is impossible to make a comparison between the results. Despite frequent and numerous uses of centrifuge device, it seems that adjusting the centrifugation speed and time has no scientific basis and in some studies, despite using similar methods detection and diagnosis of a parasite, the centrifugation speed and time vary in them (9).

It should be noted that although the result of this study was specific to the *T. galina* parasite, it can also be a starting point for studying other parasites; however, due to the special biological conditions and passing a phase or phases such trophozoite, cysts, eggs, or larvae during the evolution of the parasites, it cannot be generalized because it won't have a uniform sensitivity to centrifuge variables. Therefore, it is suggested that, along with the development and progress of the sensitive diagnostic methods, the relevant techniques including variables such as centrifugation speed and time are investigated exclusively and separately for each parasite; so that, as long as the settings of the centrifuge device as an applied diagnostic device are concerned, the references can approve the accuracy and precision of the results.

The author wishes to appreciate Vice Chancellor for Research Department of University of Medical Sciences for undertaking the costs of the project, and wishes to acknowledge Razi Herbal Medicines Research Center for providing the necessary facilities for execution of the project.

## REFERENCES

[1] Revised:Trichomoniasis in Birds.AGRI-FACTS.Practical information for alberts industry **2001**;201-206.
[2] Saif y.m,Barnes H.J,Glisson J.R,Fadly A.M,mcDougald L.R,Swayne D.E.Diseases of poultry,11th edition,Iowa state press,Ames,Iowa **2003**;1006-1008.
[3] Badparva Ebrahim, Ezatpour Behrouz ,Azami Mehdi, Badparva Masoud. *J Parasit Dis* **2015**, 39(4):720-724.
[4] Soulsby E.J.L.Helminth,Arthropods and protozoa of Domesticated Animals.7th edition.Bailliere tindall and cassel ltd .london **1982**;562-563.
[5] Ebrahim Badparva. Medical Parasitology(Helminthology and Diagnosis Methods).Kosar.**2006**.
[6] R.E Ambrosio ,D.T DE waal. *Rev.Sci.tech.off.int Epiz* **1990**;9(3):759-778.
[7] Momar Ndao.Diagnosis of parasitic Diseases old and new Approaches review Article. Hindawi publishing corporation **2009**;Article ID 278246.
[8] Christi j,schwan EV,Bodenstein LL,Sommerville JE,Van der merve. *J s Afr vet Assoc* **2011**;82(2):71-5.
[9] Ganani charitna V,Rayulu V.C,Kondaiah P.M,Srilatha ch. *J Parasites Dis* **2013**;37(2):260-263.
[10] Badparva E, Kheirandish F,Ebrahimzade F. *Asian pac J Dis* **2014**;4(1):930-934.