# The role of pseudocyst of *Trichomonas vaginalis* in transmission of infection

**Thesis**
Submitted to Faculty of Medicine, Ain Shams University
For Partial Fulfillment of Master Degree in Basic Medical Science (Parasitology)

**By**


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بسم الله الرحمن الرحيم

# وقُلِ اعْمَلُوا فَسَيَرَى اللَّهُ عَمَلَكُمْ ورَسُولُهُ والْمُؤْمِنُونَ

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**Heba Mohamed Awaed El Naggar**

# Abstract

## The role of pseudocyst of *Trichomonas vaginalis* in transmission of infection

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*T.vaginalis*, parasitic protists of the urogenital tract, display a trophozoite and a pseudocyst stage. The pseudocyst form appears under unfavorable environmental conditions when the flagella are internalized, and a true cell wall is not formed. Pseudocysts are competent to divide but their role in trichomonas life cycle has not yet confirmed. In this study, the ability of the intra-vaginally inoculated pseudocysts to induce trichomoniasis in infected mice was evaluated in comparison to the trophozoites.Pseudocyst induction was performed physically by thermal-freezing cycle method. The infectivity of pseudocyst was proved by the presence of *T. vaginalis* parasite in mice vaginal washes and by histopathological studies.In *vitro*, *T. vaginalis* trophozoites and pseudocysts were found to possess several different proteinase bands by non-denaturing gelatin-SDS-PAGE (zymography). So, *T. vaginalis* pseudocysts are active forms that can induce trichomoniasis.

**Key words**: *Trichomonas vaginalis;* pseudocysts; gelatin-SDS-PAGE; proteinase.

# Contents

# List of Abbreviations

| | |
|---|---|
| **APS** | Ammonium persulfate |
| **°C** | Degree Celsius. |
| **µg** | microgram. |
| **µl** | Microliter. |
| **µm** | Micrometer. |
| **Bis acrylamide** | bis-methylene-acrylamide C7 H10 N2 O2. |
| **CAF** | Chromosome aberration factor . |
| **CDF** | Cell detaching factor. |
| **Cm** | Centimeter. |
| **CP** | Cysteine proteinase. |
| **CPLM** | Cysteine peptone liver infusion maltose. |
| **D.P.I** | days post infection . |
| **DNA** | Deoxy ribonucleic acid. |
| **DTT** | Dithiothreitol. |
| **EDTA** | Ethyl-ene diamine tetra acetic acid. |
| **ELISA** | enzyme-linked immune sorbent assay |
| **g** | gram. |
| **HIV** | Human immuno deficiency virus. |
| **HPV** | Human papilloma virus. |
| **Hrs.** | Hours. |
| **Ig** | Immunoglobulin. |
| **Ig A** | Immunoglobulin A. |
| **Ig G** | Immunoglobulin G. |
| **IU** | International unit. |
| **$K_2HPO_4$** | Dipotassium hydrogen phosphate dibasic. |
| **kDa** | Kilodalton. |

# List of Abbreviations (Cont.)

| | |
|---|---|
| **Kg** | Kilogram. |
| **$KH_2PO_4$** | Potassium dihydrogen phosphate monobasic. |
| **L** | Liter. |
| **M** | Mole. |
| **Ma** | Milliampere. |
| **MCA** | Modified Columbia Agar. |
| **mg** | Milligram. |
| **MGJ** | Modified Glycerol jelly. |
| **Min** | Minutes. |
| **ml** | Milliliter. |
| **Mm** | Millimeter. |
| **mM** | Millimole. |
| **MW** | Molecular weight. |
| **N** | Normal. |
| **Na Cl** | Sodium chloride. |
| **Na OH** | Sodium hydroxide. |
| **$Na_2HPO_4$** | Disodium hydrogen phosphate dibasic. |
| **$NaH_2PO_4$** | Sodium dihydrogen phosphate monobasic. |
| **nm** | Nanometer. |
| **No.** | Number. |
| **Pap. smear** | Papaniculaou smear |
| **PBS** | Phosphate buffered saline. |
| **PCR** | Polymerase chain reaction. |
| **ppm** | Parts per million. |
| **rpm** | Round per minute. |

# List of Abbreviations (Cont.)

| | |
|---|---|
| **SDS** | Sodium dodecyl sulphate. |
| **SDS-PAGE** | Sodium dodecyl sulphate-polyacrylamide gel electrophoresis. |
| **St.** | Standard. |
| **STD** | Sexually transmitted disease |
| ***T. foetus*** | *Tritrichomonas foetus.* |
| ***T. vaginalis*** | *Trichomonas vaginalis.* |
| **TEMED** | Tetra methyl ethyl ene diamine. |
| **TYM** | Trypticase, yeast extract, maltose. |
| **V** | Voltage. |
| **VECs** | Vaginal epithelial cells. |

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# The role of pseudocyst of *Trichomonas vaginalis* in transmission of infection

**Protocol of**

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# Introduction

*Trichomonas vaginalis* is the most common non-viral sexually transmitted pathogen **(Guenthner et al., 2003).** The infection is prevalent in reproductive age women and is associated with vaginitis, endometritis, adnexitis, pyosalpinx, infertility, preterm birth, low birth weight, bacterial vaginosis, and increased risk of cervical cancer, HPV, and HIV infection **(Fichorova,2009)** .In men, its complications include urethritis, prostatitis, epididymitis, and infertility through inflammatory damage or interference with the sperm function **(Bennett et al., 1989).**

In addition to the trophozoite, pseudocyst is an another morphological form which is recently identified among genitourinary trichomonads **(Tasca and De carli, 2007).**

Pseudocysts were found in natural culture conditions and also under induction by hydroxyurea or cycles of cooling and warming cultures. They were studied by light microscopy, both scanning and transmission electron microscopy and by using immunofluorescence microscopy **(Benchimol, 2004)**. The ultrastructure of the trophozoite was compared to that of the pseudocyst where the latter appears under unfavorable environmental conditions when the flagella are internalized, but a true cell wall is not formed **(Pereira-Neves et. al., 2003).**

**Mariante et al.(2003)** proved that *T.vaginalis* formes pseudocyst under natural and unfavorable conditions .Morphological variability of *T.vaginalis* was determined by different environmental factors such as temperature, pH, oxygen tension, carbohydrate, and contact with other cell types **(Honigberg and Brugerollr, 1990).**The transformation of both the ellipsoid and the spherical (pseudocysts) forms into trophozoite occurred once the parasite was in direct contact with the vaginal epithelial cells (VECs)**(Arroyo et al., 1993:Arroyo and Alderete, 1995).** Besides, the human erythrocytes and microorganisms of the vaginal flora induced morphological changes on *T. vaginalis* **(Rend-on-Maldonado et al., 1998)**.

# Aim of the work

The aim of the present study is to clarify the role of *T.vaginalis* pseudocyst in transmission of trichomoniasis .

# Plan of the work:

**1. Collection of vaginal swabs:**

a- Oral consent will be taken from the patients before taking any samples.
b- Full detailed history including: age, marital status,….ect
c- Vaginal swabs and\or urine from symptomatic females in the reproductive age groups.
d- Microscopic examination with direct wet smear method using saline, iodine, eosine and methylene blue.

**2. Culture on Trypticase, yeast, and maltose (TYM) media.**

**3. Assessment of pathogenicity of both trophozoite & pseudocyst:**

**A. In vitro assessment:**

**The parasite separated from culture, will be subjected to:**

testing the proteinase activity of the cell lysate by gelatin SDS - PAGE (Sodium-dodecyl-sulphate Polyacrylamide gel electrophoresis).

**B. In vivo assessment:**

Intra-vaginal injection of the parasite in estradiol treated albino mice.

**Group I:**

Estradiol treated albino mice infected by T.vaginalis trophozoite.

**Group II:**

Estradiol treated albino mice infected by T.vaginalis pseudo cyst.

**Group III :**

**( Control** group) Estradiol treated albino mice without infection.

Then, pathological study of the three groups will be performed and compared.