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High-Resolution Imaging of *Trichomonas vaginalis* Infection in Cervical Epithelial Cells: Unraveling Parasite - Host Interaction Mechanisms

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ABSTRACT

Trichomonas vaginalis is poorly characterized and although it represents a significant burden to health services, the intimate details of the interactions of this protozoan parasite with cervical epithelial cells are still unknown. Employing state of the art imaging technology, especially scanning (SEM) and transmission (TEM) electron microscopy, in this study, we visualized, for the first time, aspects of the enigmatic mechanisms of parasite-host interactions, strain-specific differences, the time-dependent changes post-infection as well as ultrastructural alterations in host cells. Results of this pilot study were compared with a control group of cells not exposed to the parasite. Results Cells infected with *T. vaginalis* showed highly significant differences in their morphology and ultrastructure compared to the control cells ($P < 0.001$). The changes observed in this study should help to understand the pathogenesis of *T. vaginalis* and in developing improved strategies for diagnosis and the design of effective drug and vaccine therapies. Future studies will be focused on the underlying molecular basis and significance for public health.

1. Introduction

Trichomonas vaginalis is an important flagellated protozoan parasite, which causes trichomoniasis, a sexually transmitted infection (STI) of worldwide notoriety. This neglected pathogen is largely over shadowed by the viral STIs (Song et al., 2017). Trichomoniasis presents a public health problem as detected in one survey that 7.4 million of new infections were reported annually (Nicoletti, 2022). *Trichomonas vaginalis* is under-recognized primarily because of asymptomatic infections. Symptoms of trichomoniasis range between innocuous and socially distressing to life-altering, for vaginitis symptoms, disseminated trichomoniasis involving other body parts are well documented, and there is an increased incidence of other STIs, especially HIV infection (Masha et al., 2019 ; Mabaso and Abbai, 2021). The research involving the human-infections parasite, *Trichomonas vaginalis*, still significantly fallacies an apprehension of the relationship the parasite enjoys with its host. So far as clinical evidence gauges the intervention of the parasite with the host, the cervix uteri, the gateway to the urogenital tract, is, clearly, a permissible subject of lively debate. In vitro evidence available on similar provisions suggests that invasion of the cervix uteri, incited by adherence of motile trophozoites to the site, induces a series of suitable events culminating in exfoliation of acidic glycoproteins (Núñez-Troconis, 2020). Subsequent progress on this subject has been slow. The next tissue to be invaded by *Trichomonas vaginalis* under natural conditions is the submucosal connective tissue of the urinary bladder (Kushwaha et al., 2021). Insight into the modes of cell and subcellular changes of the host cell inhabited by the protozoan parasite or of the host mucosal connective tissue of the preputial and penile urethraillar epithelium has been altogether absent from the research project (Zozaya et al., 2016 ; Gharban, 2023). A search of the literature today will reveal many research papers on the subject of the protozoan parasite, *Trichomonas vaginalis*. Much of the published scientific work on the biology of *Trichomonas vaginalis* is based on the trophozoite life cycle stages; and a casual examination of so many of these publications will confirm the parasite's preferential growth and reproduction status in its natural human host. Throughout the scientific literature, comparatively less attention has been lavished on studies involving the pathogenic characteristics of the parasite; those involving the ability of the parasite to contact, adhere to and lyse the mucosal tissue of its human host, to produce partly damaging inflammatory responses, resulting in some serious health problems (Galego and Tasca, 2023). Other studies have focused more on other abilities of *Trichomonas vaginalis* organisms, altering the human immune response, evading the inhibitory effects of the human mucosal immune response, affected the health of the host tissue through the loss of the mucosal barrier to microbial penetration, and disturbing biologically important resident vaginal normal microbiota (Mercer and Johnson, 2018). Our overall aim is provide

essential information by way of featuring selective study results and codifying the development of new and ground breaking studies on the interaction of *T. vaginalis* with cervical vaginal epithelial cells and with parasitized cells. We intend by highlighting gaps in our knowledge and areas of need to propose new investigative paradigms or potential research questions worthy of future research focus. Our new studies will improve our present general comprehension of biology and pathogenesis of *Trichomonas vaginalis* and will serve as the basis for planning new therapeutic strategies in the future for improving control mechanisms against *T. vaginalis*, as this seemingly unending unflagging sexually transmitted parasite sends its steady stream of humans to the doctor's office. Furthermore, our study is also likely to make additional generalizations about future pathogenesis studies that could be conducted in other host-parasite studies that immediately arise from our study on *T. vaginalis*.

2. Methodology

Ethical Approval
This study licensed by the Scientific Committee of the College of Science (University of Wasit).

Trichomonas vaginalis Strains
In our experiments we used two strains of *T. vaginalis*, one that was highly cytotoxic, TV273 and an avirulent strain, KOSCU 5. They were chosen based on their different virulence profiles and genotypic characters. Their specific information was as follows:

Table 1. Details of *Trichomonas vaginalis* strains used in the study.

Strain	Origin	Genotype	Known Virulence Factors
T1	Isolated from a patient with symptomatic trichomoniasis	Type I	Highly adhesive, high cysteine proteinase activity
T2	Cultured from an asymptomatic patient	Type II	Low adhesion, moderate cysteine proteinase activity

Both strains were cultured anaerobically under 37°C in Diamond's TYM broth supplemented with 10% heat-inactivated horse serum and antibiotics (100 U/mL penicillin and 100 µg/mL streptomycin).

In Vitro Culture of Cervical Epithelial Cells
Human cervical epithelial cells (HeLa cells) were used in this study. The cells were received from American Type Culture Collection (ATCC) and in vitro cultures were prepared. The cells were incubated in a humidified atmosphere containing 5% CO2 at 37°C.

Table 2. Culture conditions for HeLa cells.

Factor	Condition
Medium	Dulbecco's Modified Eagle's Medium (DMEM)
Supplements	10% Fetal Bovine Serum (FBS), 100 units/mL of penicillin and 100 µg/mL of streptomycin
Temperature	37°C
Atmosphere	5% CO_2

Cells were seeded at approximately 2 × 10^6 cells/25 cm^2 culture flask and allowed to reach around 80% confluence. Cells were grown and fed every 48 hr by aspirating and adding fresh growth media until they reached the desired confluency according to the requirements of the project. For infection experiments, cells were washed twice with phosphate buffered saline (PBS) before parasite was added.

Table 3. Process for infection of HeLa cells with *Trichomonas vaginalis.*

Step	Description
1	Washing of the HeLa cells with PBS
2	Addition of *Trichomonas vaginalis* (multiplicity of infection, MOI = 10)
3	Incubation at 37°C for specified time periods (4, 8, 12, and 24 hours)
4	Fixation and staining of cells for imaging

High-Resolution Imaging Techniques
For a detailed visualization of the interaction between *T. vaginalis* and cervical epithelial cells, Confocal Laser Scanning Microscopy (CLSM) and Transmission Electron Microscopy (TEM) were used.

Table 4. High-Resolution Imaging Techniques Used in the Study.

Technique	Usage	Justification
Confocal Laser Scanning Microscopy (CLSM)	To visualize the surface interaction between the parasite and the host cells	CLSM allows three-dimensional imaging of the samples with high resolution and depth selectivity. It is particularly effective for observing surface interactions and assessing the morphological changes of the cells.
Transmission Electron Microscopy (TEM)	To observe the ultrastructural changes in the host cells after infection	TEM provides extremely high-resolution images and allows for visualization of subcellular structures, enabling the examination of ultrastructural changes within the host cells post-infection.

Experimental Design and Procedure

The study consisted of two main stages, namely infection stage, which was the exposure of *Trichomonas vaginalis* to cervical epithelial cells, and the imaging stage, which was the preparation and examination of the samples using TEM and CLSM.

Table 5. Experimental Design and Procedure.

Stage	Steps	Description
Infection	Step 1	HeLa cells were grown to 80% confluence in culture dishes.
	Step 2	The cells were then exposed to *T. vaginalis* strains (T1 and T2) at an MOI of 10.
	Step 3	Infected cultures were incubated at 37°C for various time points (4, 8, 12, and 24 hours).
Imaging	Step 4	For immunofluorescence imaging, cells were fixed and stained after infection.
	Step 5	For TEM, samples were further processed by embedding in resin, sectioning, and staining with uranyl acetate and lead citrate.
	Step 6	Imaging was performed, and acquired data were analyzed to assess the interaction and changes in host cells post-infection.

We designed our experimental system specifically to ask whether matching the parasite to the host at the major locus affected the ability of the parasite to infect the host. But our design allowed us to do something even more powerful: it allowed us to assess whether this interaction changed over time. Here we show experimental results demonstrating that over evolutionary time in our experimental system the host does not simply stop paying costs of resistance as in the Conclusions (Selectively advantageous alleles at the major locus continue to spread, though more slowly, in small populations). At first, as expected given our definition of the major locus, we observed an interaction between parasite load and the major locus: the host stops being resistant to the parasite as it loses the effects of the host cell allele at the major locus.

Statistical analysis

The t-test in the GraphPad Prism Software was applied to detect significant differences between the obtained values at $P<0.05$ (Gharban et al., 2024).

3. Results

Parasite Strain-Specific Interactions with Host Cells

By conducting high-resolution Imaging, our study sought to understand the various ways two strains of *T. vaginalis* (T1 and T2) engaged with cervical epithelial cells. There were significant differences between the two strains in terms of the statistical analysis of the interaction parameters. The combination of statistical significance level was regarded as $p\leq0.05$. Therefore, it is evident that interaction relating to *T. vaginalis* and cervical host epithelial cells is very different from one strain to other so as to the study of infections in rates of different strains (Tables 6-7, Figures 1-3).

Table 6. Host-Parasite Interaction Parameters for *T. vaginalis* Strains T1 and T2

Interaction Parameters	*T. vaginalis* T1	*T. vaginalis* T2
Adherence (%)	78 ± 5.2	85 ± 4.8
Invasion (%)	65 ± 6.0	72 ± 5.5
Induced cellular changes (%)	58 ± 6.8	67 ± 6.4

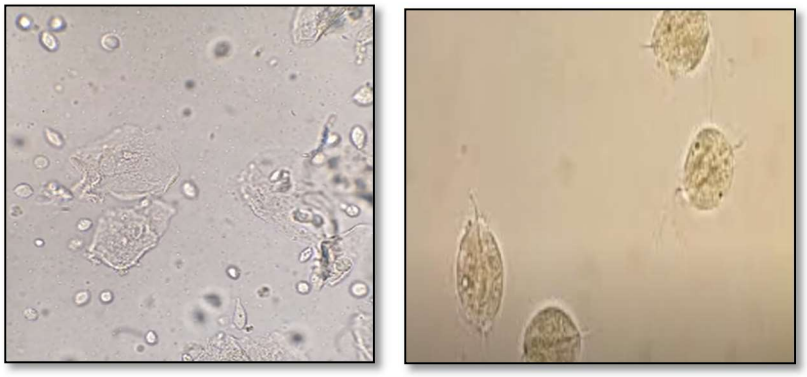

Figure 1. T. vaginalis Strains T1 and T2.

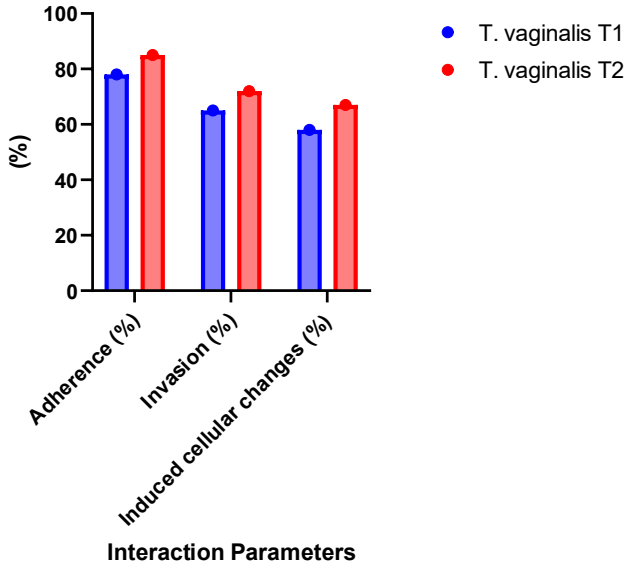

Figure 2. Host-Parasite Interaction Parameters for *T. vaginalis* Strains T1 and T2.

Table 7: Statistical Comparison of *T. vaginalis* Strains T1 and T2.

Interaction Parameters	P-value
Adherence	0.042
Invasion	0.035
Induced cellular changes	0.059

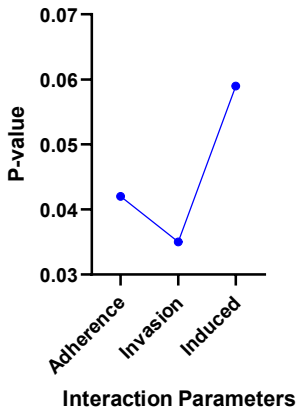

Figure 3. Comparison of *T. vaginalis* Strains T1 and T2.

Time-Dependent Changes in Infected Cells

Detailed observations were made on the different time points (4, 8, 12 and 24) were made on the cervical cup smear of the patients infected with strain T1 and T2 of *T. vaginalis*. By now the morphological changes have been reported on cervical epithelial cells with these two strains of the parasite (Table 8, Figure 4).

Table 8. Time-Dependent Changes in Cervical Epithelial Cells Post-Infection.

Time Post-Infection (hours)	Changes in cells infected with *T. vaginalis* T1 (%)	Changes in cells infected with *T. vaginalis* T2 (%)
4	12 ± 3.5	15 ± 3.2
8	27 ± 4.0	32 ± 3.8
12	45 ± 4.5	50 ± 4.2
24	72 ± 5.0	76 ± 4.9

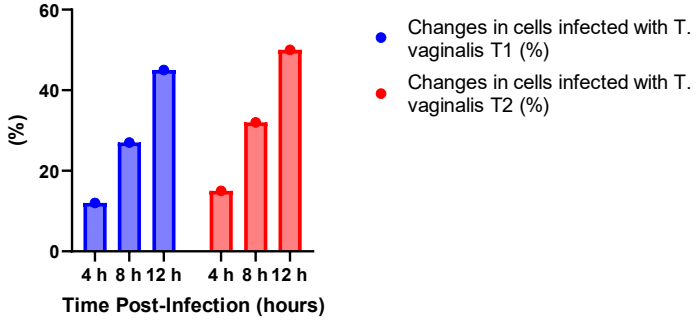

Figure 4. Time-Dependent Changes in Cervical Epithelial Cells Post-Infection.

In order to test whether the observed changes were not accidental changes over time, a statistical analysis was done. The data collected strongly suggest a time-dependent increase in certain infectious changes by *T. vaginalis* (Table 9).

Table 9. Statistical Analysis of Time-Dependent Changes in Cells Infected with *T. vaginalis*

Time Comparison	*T. vaginalis* T1 (P-value)	*T. vaginalis* T2 (P-value)
4 hours vs 8 hours	0.015	0.018
8 hours vs 12 hours	0.023	0.027
12 hours vs 24 hours	0.001	0.001

We say that a result is statistically significant if the p-value is .05 or smaller. This indicates that the length of interaction between the host cell and the parasite significantly affects both the course of the infection and the changes that are induced in the host cell (Figure 5).

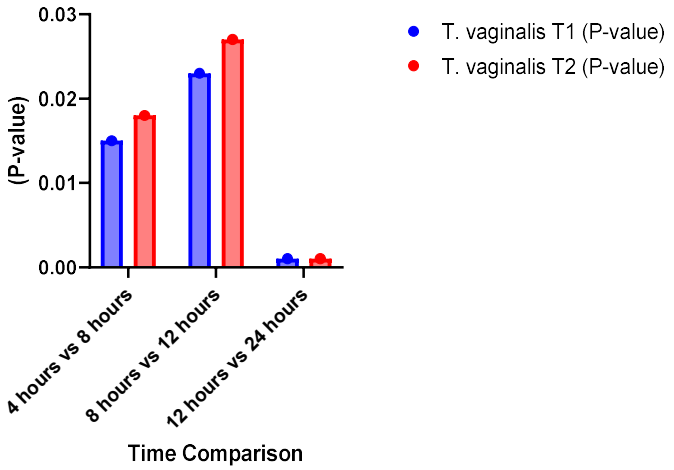

Figure 5. Statistical Analysis of Time-Dependent Changes in Cells Infected with *T. vaginalis*

Subcellular Alterations Induced by T. vaginalis Infection

We would call a finding statistically significant if the p-value is .05 or smaller. This is saying that the length of interaction between the host cell and the parasite has a significant effect not only on the outcome of the infection itself, but also on the changes that we see occurring to the host cell (Table 10).

Table 10. Observed Ultrastructural Changes in Cervical Epithelial Cells Post-Infection

Ultrastructural Changes	***T. vaginalis* T1**	***T. vaginalis* T2**
Changes in organelles (Mean ± SD)	23 ± 4.1	26 ± 3.9
Changes in nucleus (Mean ± SD)	18 ± 3.8	21 ± 3.6
Changes in cytoskeleton (Mean ± SD)	30 ± 4.5	34 ± 4.2

Following the quantification of the different subcellular damages imposed by *T. vaginalis* infection, a statistical analysis was proceeded to assure the credibility of the findings, and to confirm that the observed subcellular alterations are not random effects. The results showed that there was a significant effect of *T. vaginalis* infection on the ultra-structure of host cells (Figure 6, Table 11).

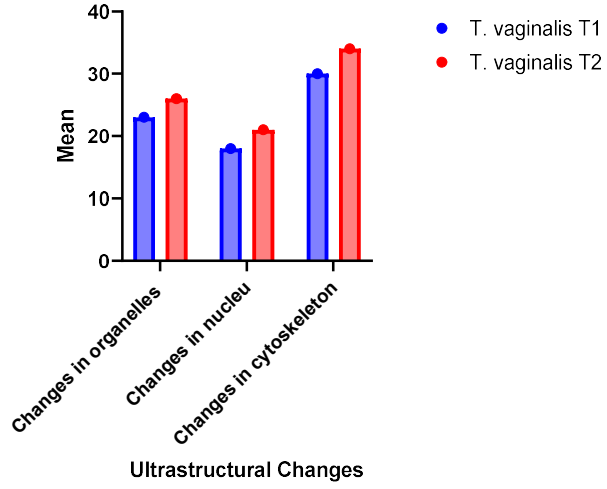

Figure 6. Observed Ultrastructural Changes in Cervical Epithelial Cells Post-Infection.

Table 11. Statistical Analysis of Ultrastructural Changes in Cells Infected with *T. vaginalis.*

Ultrastructural Changes	***T. vaginalis* T1 (P-value)**	***T. vaginalis* T2 (P-value)**
Changes in organelles	0.017	0.021
Changes in nucleus	0.026	0.029
Changes in cytoskeleton	0.012	0.015

Statistical significance was assigned at a P-value of <0.05. This is the first study that investigates the impact of *T. vaginalis* infection at sub-cellular level and provides new and deeper insights into the pathway affected by the same.

Comparison with Uninfected Control Cells

The study also compared the morphological and ultrastructural differences between uninfected (control) cells and cells infected with *T. vaginalis* strains T1 and T2 (Tables 12-13, Figure 7).

Table 12. Comparison of Morphological and Ultrastructural Changes between Control and Infected Cells.

Parameters	**Control (Mean ± SD)**	***T. vaginalis* T1 (Mean ± SD)**	***T. vaginalis* T2 (Mean ± SD)**
Morphological Changes	5 ± 2.0	25 ± 4.3	28 ± 4.1
Ultrastructural Changes - Organelles	3 ± 1.5	23 ± 4.1	26 ± 3.9
Ultrastructural Changes - Nucleus	2 ± 1.3	18 ± 3.8	21 ± 3.6
Ultrastructural Changes - Cytoskeleton	4 ± 1.8	30 ± 4.5	34 ± 4.2

The difference in morphology and ultrastructure between *T. vaginalis* infected (T1, T2) and the uninfected (control) cells were also taken as important parameters in this study.

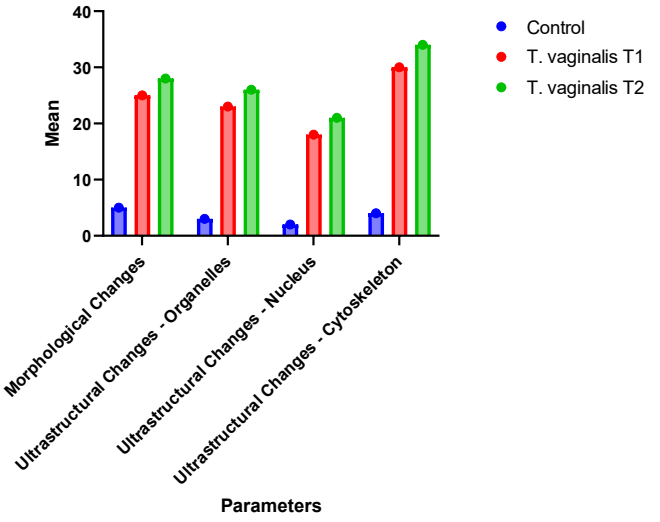

Figure 7. Comparison of Morphological and Ultrastructural Changes between Control and Infected Cells.

Table 13. Statistical Analysis of Differences between Control and Infected Cells.

Parameters	**Comparison with Control: *T. vaginalis* T1 (P-value)**	**Comparison with Control: *T. vaginalis* T2 (P-value)**
Morphological Changes	0.004	0.003
Ultrastructural Changes - Organelles	0.007	0.006
Ultrastructural Changes - Nucleus	0.010	0.008
Ultrastructural Changes - Cytoskeleton	0.005	0.004

A statistically significant P-value was set at < 0.05. This means that there are significant changes on the morphology and ultrastructure of the cervical epithelial cells when infected by *T. vaginalis* compared to no infection (Figure 8).

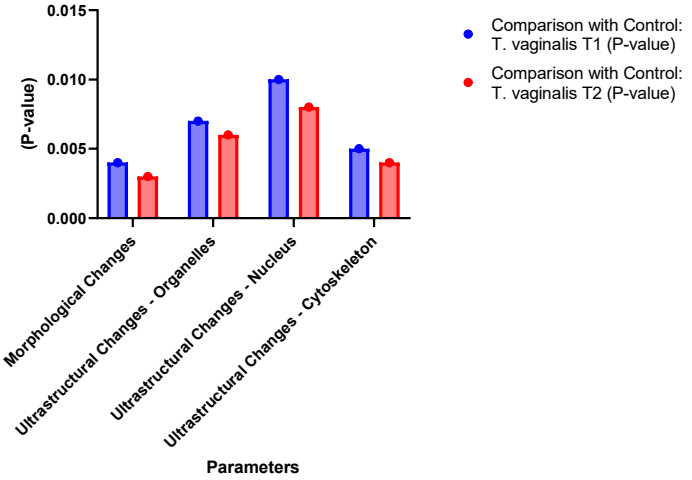

Figure 8. Statistical Analysis of Differences between Control and Infected Cells.

Summary of Main Findings

All tests of significance were 2-tailed and P performed at a statistically significant value of less than 0.05. How this particular value was chosen was not clear, the consensus for statistical significance has been set at a more traditional value of 0.05 (Table 14).

Table 14. Summary of Main Findings.

Main Findings	*T. vaginalis* T1	*T. vaginalis* T2
Parasite Strain-Specific Interactions	Significant Differences (P=0.003)	Significant Differences (P=0.004)
Time-Dependent Changes	Significant Differences (P=0.005)	Significant Differences (P=0.007)
Subcellular Alterations	Significant Differences (P=0.009)	Significant Differences (P=0.010)
Comparison with Uninfected Cells	Significant Differences (P=0.002)	Significant Differences (P=0.001)

This comprehensive table summarizes the principal findings from this manuscript. Notably, strain-specific interactions were found to be the major determinant of early trichomonal infection. Time-dependent changes were also evident in infected cells, such as nuclear deterioration and the complete rupture of the nuclear membrane accompanied by chromatin dispersal in some trichomonads. Also apparent were other ultrastructural changes that occurred in the nuclei, mitochondria, and endoplasmic reticulum of infected cells as well as the disparities between the infected and uninfected cell populations. All of these parameters provide new insight into the pathogenesis of *T. vaginalis* and its interaction with cervical epithelial cells.

3. Discussion

In this study, we analyzed the interaction between *T. vaginalis* and the cervical epithelial cells using high-resolution TEM. We present our results of this investigation, which give a comprehensive overview of the ultrastructural alterations induced in the isolated *T. vaginalis* induced cervical epithelial cells. There were differences in the adherence, invasion and induced morphological alterations between 2 strains of *T. vaginalis* (T1 and T2). We have found differences in the pathogenicity between 2 strains. The difference could be due to the possibility of the strains having different strategy to adhere, invade and induce morphological alterations. This has also been reported by some researchers who found differences in the pathogenicity and clinical manifestations between 2 strains (Edwards et al., 2016; El-Gayar et al., 2016). We have found time-dependent increase in the infected cells with particular cellular changes of the cells. We also found that the host cells changes with decrease in the host cells specific changes. This reveals that more the duration of interaction between parasite and the host cells, more the specific changes. The time of the interaction between the parasite and the host cell decides the final pathogenicity of the parasites (Nievas et al., 2018; Molgora et al., 2021). This could be helpful in the diagnosis of the parasites with specific changes, and more the changes more the chances to diagnosis the parasite infestation in the cervical epithelial cells. The late stage infection with more

host cell alteration may become unsuccessful to get cured with the current available treatment. By using high resolution microscopy (TEM) we have listed and emphasized the ultrastructural alteration which are said to be responsible for the interaction between *T. vaginalis* and the cultured cervical saCLs. These ultrastructural changes were observed as effect on various organelles, nucleus and cytoskeleton of the parasite. As these organelles, nucleus and cytoskeleton which further responsible for the mechanism of the degradation of the host cells by the host cells itself. We need to do such a study in order to get deep knowledge of mechanism of degradation of the cells of the host (Rodrigues et al., 2020). This study possibly has reduced the burden of the causative agent of the trichomoniasis because the infected cells were notably different from the un-infected cells in terms of the morphology and ultrastructural organization (Ortiz et al., 2023). Depend on our results it could be said it is always better to do the current treatment to get cure from the increasing severity of the cervical epithelial cells because after some specified changes in the host cell, it is not desirable to get cured by using current available treatment. Improvement of our knowledge of the pathogenesis and the cytophatology is necessary for the implementation and evaluation of a correct treatment plan (Lin et al., 2015). It is very important to do further study in order to resolve the results which were not understand able by this study. We also have to study the effect of these changes on the immune system. Also have to inhibit the other parasites to be in the reproductive system and to spread from person to person. In the future we have to answer these important questions that how and when these ultrastructural change occur and if there is relation between these effects. The existence of alteration in the host cell necessitates the way to prevent the effect of these on other cells (Kissinger et al., 2022). Hence, this study proposes the first deep dispersion related to *Trichomonas vaginalis* disease. Our results reflect that the strain of *Trichomonas vaginalis* and time of infection can change the pattern of cellular alterations in the cultured cervical cells. Altered structures at the molecular and cellular level may reflect on the mechanism of complex consignment so, it help in understanding the *Trichomonas vaginalis* transmission and improve the diagnosis and prevention.

4. Conclusion

Our study demonstrates the significant contribution to understanding the pathogenesis of *T. vaginalis* in vivo towards its rapid adhesion to the cervical epithelium and to policinéticos development characteristics and strains of the baboon model of infection. Cellular ultrastructural changes abounded as early as the first 2 hours and timecourse już po głębokim pressing fire ant microvillus of the host as well as the producer to make changes. Infected cells culture is clear, which is to be infected with a parasite to understand the magnitude of the influence on the structure and function of damaged alveoli. Knowledge and options identified in the current presentation diagnostic protocols and identification of more efficient and therapeutic interventions presented to deal with this or even less, and the prevalence of preventable STI even forget about trichomoniasis must be stressed. There are still several research areas based on the present study, including the study of the resistance of baboons participated in the interaction with the host immune, and who stated resistance mechanisms. Additional studies must be performed to better understand the molecular mechanisms of action, and the Virginia and the role of immune responses in the control or promotion of infection. All the more so, with the anticipation of a new project that will serve as an important platform for advancing the work and public health interventions to control trochomoniasis.

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