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Trichomonas vaginalis infection in symbiosis with Trichomonasvirus and *Mycoplasma*

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Abstract

Trichomonas vaginalis is a protozoan with an extracellular obligatory parasitic lifestyle exclusively adapted to the human urogenital tract and responsible for nearly a quarter billion sexually transmitted infections worldwide each year. This review focuses on symbiotic Trichomonasvirus and mycoplasma carried by the protozoan, their molecular features and their role in altering the human vaginal microbiome and the immunopathogenicity of the parasite. Improved diagnostics and larger clinical interventional studies are needed to confirm the causative role of protozoan symbionts in the variable clinical presentation of trichomoniasis and its morbid sequelae, including adverse reproductive outcome, susceptibility to viral infections and cancer.

Keywords: Trichomoniasis; Endosymbionts; dsRNA viruses; *Totiviridae*; *Mycoplasma hominis*; *Candidatus Mycoplasma girerdii*; Microbiome; Human vagina

1. Introduction

Protozoan genera known to carry endobiont viruses that can only propagate within the protozoan host include *Babesia*, *Cryptosporidum*, *Eimeria*, *Entamoeba*, *Giardia*, *Leishmania*, *Naegleria*, *Plasmodium* and *Trichomonas* [1]. While these viruses are presumably non-infectious to the human/animal host, recent evidence suggests some may significantly influence the outcome of parasitic disease by modifying immune responses to the protozoan parasite [2–4]. Protozoan parasites that carry symbiont microorganisms that are capable of multiplying in the vertebrate host and that cause an infectious disease are more rarely described. *Trichomonas vaginalis* falls within both categories of protozoan pathogens. It has adapted to symbiosis with double-stranded RNA (dsRNA) endobiont viruses, recently classified by the International Committee on Taxonomy of Viruses as Trichomonasvirus genus within the Family *Totiviridae*, as well as with eubacterial *Mycoplasma* species, with *Mycoplasma hominis* as the best studied representative. This review will focus on *T. vaginalis* and its toolbox of symbionts as emerging key players in human disease. We believe that understanding the molecular features of the symbiont infections and their interactions with the human host is essential for improvement of the diagnostics and therapeutics of this parasitic disease.

T. vaginalis is an extracellular, obligatory sexually transmitted parasite, exclusively adapted to the epithelial lining of the human vagina, the uterine cervix and the male and female urethra. It causes over 220 million cases of trichomoniasis each year, which is more than the most prevalent bacterial sexually transmitted infections taken together [5]. The

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infection is often asymptomatic and, when present, symptoms range widely from itching to burning, dyspareunia and malodorous discharge [6]. Trichomoniasis is associated with persistence of most carcinogenic HPV types, cervical and prostate cancer and higher risk of HIV and HPV infection, and is especially damaging to reproductive health and pregnancy (reviewed in [6]).

In culture supernatant, the parasite is pear-shaped, measuring 7–23 × 5–10 microns, and in the trophozoite state it can be almost half the size of the host epithelial cells (Fig. 1). It swims using 5 flagella – four anterior and one embraced by an undulating membrane across its antero–posterior axis. It can survive on wet surfaces outside the human body for only a limited amount of time.

2. Symbiotic species prevalent among *T. vaginalis* isolates across the globe

2.1. Trichomonasvirus

The presence of long, linear dsRNA molecules in many strains of *T. vaginalis* was first reported in 1985, followed shortly by evidence of their association with virus-like particles and their recognition as *T. vaginalis* viruses (TVVs) [7]. Significant progress has been made since then, especially in establishing the molecular and structural characteristics of the virus. The genome is monosegmented, with plus-strand RNA (viral mRNA) containing two open reading frames encoding the coat protein (CP) and the RNA-dependent RNA polymerase (RdRp) [8]. Like most other members of *Totiviridae*, TVV is believed to lack virion-associated machinery for active cell entry and is transmitted from one parasite to another during cytokinesis and possibly sexual reproduction inferred from genetic evidence (reviewed in [9] and [10]). Thus far, four different TVV species (TVV1, 2, 3 and 4) have been identified in the genus Trichomonasvirus by phylogenetic and genomic sequence analysis, and complete genome sequences of all four species have been deposited in GenBank and assigned accession numbers as published [8,11,12]. Recently, Parent et al. described the 3D-structure of TVV-1, making it the fourth member of the Family *Totiviridae* with reported crystallographic structure, next to the prototype Totivirus *Saccharomyces cerevisiae* virus L-A (ScV-L-A), which encodes toxins in the killer yeast *S. cervevisiae*, the *Helminthosporium victoriae* virus-190S (HvV190S), which inhabits a number of pathogenic fungi and protozoa in plants, and the infectious myonecrosis virus (IMNV), which inhabits the penaeid scrimp [9]. One of the most interesting features determined by cryo-transmission electron microscopy in this study was that the TVV-1 capsid has unusually large channels that may allow the dsRNA genome to escape the virions and interact directly with the human host as an immunity modifier once released from the protozoan host and taken up by the human cells, where they engage pathogen recognition receptors without causing a productive infection [3].

The reported infection rate of TVVs in different clinical isolates of *T. vaginalis* varies depending mostly on detection methods and limitations of small sample size (Table 1). A few studies reported low prevalence of *T. vaginalis* dsRNA virus (14–20%) in isolates from Korea, Iran, Egypt and the Philippines [13–16]; however, most other studies reported high infection rates of 40–100% around the globe [3,17–24], suggesting that variations among studies may be driven by technical factors and/or clinical and socio-economic covariates, rather than by geographic and racial/ethnic differences. In each of the listed studies in Table 1, TVV genomic RNA was detected by gel electrophoresis of total nucleic acid extracts, electrophoresis of RNA or dsRNA extracts and RT-PCR. Other methods of TVV detection, still pending a broader validation, include immunodetection with TVV-specific antibodies [25] and nucleic acid microarrays to detect TVV RNAs [26]. None of these methods has yet been adapted as a standard clinical diagnostic test. Over 100 strains representing the four TVV species have been described to

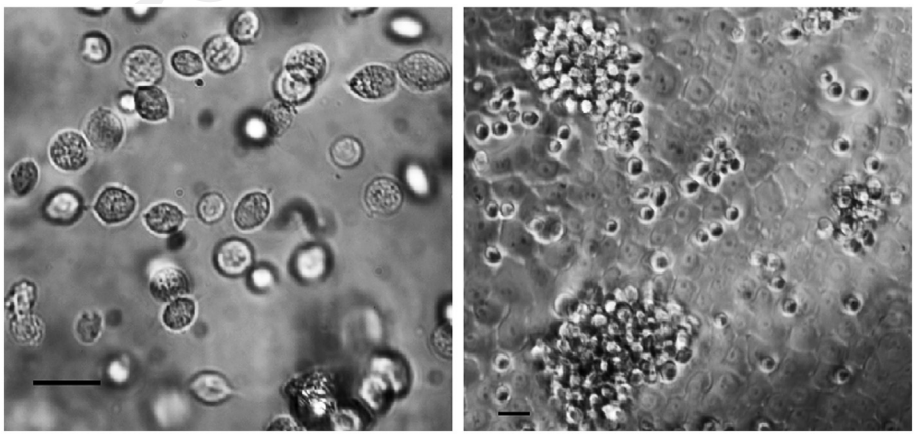

Fig. 1. Light microscopic images of *T. vaginalis* taken by phase invert microscopy. Left image depicts free-swimming parasites isolated from a vaginal swab and placed into culture medium. Right image depicts in vitro infection of human vaginal epithelial cells with *T. vaginalis*. The epithelial cells are grown in a monolayer. The parasites appear over the vaginal epithelial surface as single organisms or in swarms of many closely assembled bodies. Size bars in each image represent 15 microns.

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