Trichomonas vaginalis
(protist: flagellate)

Overview

Protists are single-celled organisms with membrane-bound nuclei (eukaryotes). Flagellates are protists that swim using one or more flagella (undulipodia); each arising from a small centriole (basal body, kinetosome) and having a microtubular axoneme core (2+9 configuration). Rather than forming a monophyletic group, flagellates are divided into several disparate groups: metamonads (amitochondriate flagellates), heteroloboseans (amoeboflagellates), euglenozoans (euglenids and kinetoplastids), stramenopiles (heterokonts), alveolates (dinoflagellates) and cercozoans (biflagellates). The metamonads comprise fornicates (diplomonads), parabasalians (trichomonads, hypermastigids, retortamonads) and preaxostylans (oxymonads). Parabasalid flagellates are anaerobic amitochondriate protists which have distinctive parabasal bodies (dictyosomes) adjacent to flagellar basal bodies (kinetosomes) and an axostyle-pelta complex providing structural support. Trichomonads are a major constituent group and most have 4-6 apical flagella, one being recurrent and often forming an undulating membrane supported by a costa. Most species have simple life cycles with longitudinal binary fission of motile-flagellated or rounded tissue-phase cells (only a few species form cysts). Many *Trichomonas* spp. have been reassigned to different genera based on the number of free anterior flagella e.g. *Tritrichomonas*, *Tetratrichomonas* and *Pentatrichomonas* having 3, 4 and 5 anterior flagella respectively. Confusingly, the name *Trichomonas* has been retained for some species with 4 anterior flagella (mainly those in man, some rodents and birds). Most trichomonad species are endocommensals in mammals, birds, reptiles and insects but several species are parasitic in the alimentary or urogenital tracts of man and domestic animals.

Classification:

Domain:	Eukaryota (membrane-bound nucleus)
Supergroup:	Excavata (with conspicuous ventral feeding groove)
Group:	Metamonad (amitochondriate flagellates with karyomastigonts)
Phylum:	Parabasalia (anaerobic flagellates with parabasal body supporting Golgi cisternae or dictyosome, trichomonads, hypermastigids, retortamonads)
Class:	Trichomonadea (single mastigont, comb-like structure absent, infrakinetosomal body absent)
Order:	Trichomonadida (lamelliform undulating membrane, B-type costa)
Family:	Trichomonadidae (5-6 flagella, cone-like axostyle)
Genus:	*Trichomonas* (parasites/commensals in tubular organs of vertebrates)
Species:	*T. vaginalis* (causes vaginitis in humans)

Parasite biodiversity and host range: Protists are unicellular eukaryotes that move using undulipodia (flagella or cilia), pseudopodia (false-feet) or a unique gliding motion. Flagellated species have one or more flagella with an internal microtubular core (in a characteristic 2+9 configuration comprising 2 single central microtubules and 9 peripheral doublets) anchored to a submembranous protein structure (known variously as a centriole, basal body, kinetosome or blepharoplast). Many types of flagellated cells have been described and recent phylogenetic studies have classified them into several disparate groups: including the metamonads (amitochondriate flagellates), heteroloboseans (amoeboflagellates), euglenozoans (euglenids and kinetoplastids), stramenopiles (heterokonts), alveolates (dinoflagellates) and cercozoans (biflagellates). While most flagellated protists are free-living organisms swimming and feeding in aquatic environments, representatives of several groups have developed symbiotic relationships with various hosts; some being endoparasitic in vertebrates (notably anaerobic metamonads in tubular organs, and heterotrophic euglenozoans occurring in blood or tissues), and some being parasitic in invertebrates (alveolates in crustacean tissues) (representatives tabulated below).

Higher taxonomy	Class or order	Family	Genera	Hosts (tissues)	Trans-mission*
Supergroup: Excavata (with conspicuous ventral feeding groove)					
Group: Metamonad (amitochondriate flagellates with karyomastigonts)					
Phylum: Fornicata (diplomonads)	Order: Diplomonadida (1-2 karyomastigonts)	Hexamitidae (2 karyomastigonts with binary axial symmetry)	*Giardia*	vertebrates (gut)	direct (f-o)
			Hexamita *Spironucleus*	vertebrates (tissues)	direct (f-o, w)
Phylum: Parabasalia (with parabasal body)	Order: Trichomonadida (3-5 anterior flagella plus recurrent flagellum)	Monocercomonadidae (costa absent, most without undulating membrane)	*Histomonas*	birds (gut, liver)	direct (f-o)
			Dientamoeba	vertebrates (gut)	direct (f-o)
		Trichomonadidae (stout axostyle, costa, undulating membrane)	*Trichomonas*	vertebrates (urogenital tract, gut)	direct (f-o, v)
		Cochlosomatidae (anterior adhesive disc)	*Cochlosoma*	birds (gut)	direct (f-o)
Group: Discoba (diverse group supported robustly by molecular studies)					
Phylum: Euglenozoa (flagella inserted in anterior pocket, heterotrophs, autotrophs)	Class: Kinetoplastea (heterotrophs, with extranuclear DNA (= kinetoplast) associated with mitochondrion)	Ichthyobodonidae (flagellar pocket continues as groove)	*Ichthyobodo* (= *Costia*)	fish (gills, skin)	direct (w)
		Parabodonidae (epizoic or endozoic)	*Cryptobia*	fish (gills, skin)	direct (w)
			Trypanoplasma	fish (blood)	indirect (v-b)
		Trypanosomatidae (monogenetic forms in insects/plants, digenetic forms in vertebrates & arthropods)	*Trypanosoma*	vertebrates (blood, tissues)	indirect (v-b)
			Leishmania	vertebrates (blood, tissues)	indirect (v-b)
Supergroup: SAR (Stramenopiles + Alveolata + Rhizaria) (3 groups unified by molecular studies)					
Group: Alveolata (with cortical alveoli)					
Phylum: Dinoflagellata (with unique mesokaryotic nuclei)	Order: Blastodiniales (uninucleate trophonts with chloroplasts)	Oodiniaceae (trophont with rhizoid-like invasive organelle)	*Amyloodinium* *Crepidoodinium* *Piscinoodinium*	fish (skin)	direct (w)
	Order: Syndiniales (multinucleate plasmodial trophonts)	Syndiniaceae (without chloroplasts)	*Haematodinium* *Ichthyodinium*	crustaceans, fish (tissues)	direct (w)
Phylum: Perkinsozoa (parasitic)	Order: Perkinsorida (released trophonts form biflagellated zoospores)	Perkinsidae (incomplete conoid)	*Perkinsus*	gastropods, bivalves (tissues)	direct (w)

*f-o = faecal-oral transmission; v-b = vector-borne transmission, w = water-borne transmission; v = venereal transmission

Metamonads are a group of excavates (with ventral feeding groove) that have several subcellular elements associated with their flagella forming a unique mastigont (an ultrastructural complex of organelles and cytoskeletal fibrils (incl. dictyosomes (Golgi bodies), centrioles (basal bodies) and a microtubular axostyle)). The metamonads comprise fornicates (diplomonads), parabasalians (trichomonads, hypermastigids, retortamonads) and preaxostylans (oxymonads). Most metamonads are amitochondriate but have retained reduced organelles of mitochondrial origin (fornicates containing mitosomes while parabasalians possess hydrogenosomes). Members of the phylum Parabasalia typically possess parabasal bodies adjacent to Golgi bodies (dictyosomes), and have microtubular arrays forming a conspicuous pelta-axostyle complex (cap-like pelta and a cone- or tube-like longitudinal axostyle). Six parabasalid classes are currently recognized on the basis of morphological, biological and molecular phylogenetic studies. Cells in three classes (Trichomonadea, Tritrichomonadea, Hypotrichomonadea) bear single mastigonts (set of kinetosomes (basal bodies) and associated appendages – ancestral unit comprising 4 kinetosomes) with flagella arranged in an anterior tuft, but many have one recurrent flagellum forming an undulating membrane (lamelliform or rail-type) supported by a costa (A- or B-type) and sometimes a basal comb-like structure and/or infrakinetosomal body. Many species are symbiotic (mutualists, commensals or parasites) in animals, although some are free-living in moist habitats. Most species have simple life cycles with longitudinal binary fission of motile-flagellated or rounded tissue-phase cells (only a few species form cysts). Cells in another three classes (Cristamonadea, Trichonymphea, Spirotrichonymphea) have more complex structures, often with multiple mastigonts bearing hundreds to thousands of flagella. Most were previously assigned to the now-defunct group Hypermastigida and they are primarily found as symbionts (mutualists) in insects (mostly termites).

Trichomonad taxonomy can be very confusing as many *Trichomonas* spp. have now been reassigned to sister genera based on the number of anterior flagella e.g. *Tritrichomonas*, *Tetratrichomonas* and *Pentatrichomonas* having 3, 4 and 5 anterior flagella respectively. Confusingly, the name *Trichomonas* has been retained for some species with 4 anterior flagella (mainly those in man, some rodents and birds). Recent ultrastructural and molecular biological studies have also led to the placement of the genus *Tritrichomonas* into a separate class (Tritrichomonadea) as the cells contain unique comb-like structures and infrakinetosomal bodies at the bases of their recurrent flagella (both lacking in members of the class Trichomonadea). Several genera (*Hypotrichomonas* and *Trichomitus*) were assigned to another class (Hypotrichomonadea) as their cells contained comb-like structures but lacked infrakinetosomal bodies.

Family	**Key characters to 'trichomonad' families in vertebrates***						**Representative genera**
	Number of flagella	**Undulating membrane**	**Costa**	**Axostyle**	**Comb-like structure**	**Infra-kinetosomal body**	
Class Tritrichomonadea (uninucleate to binucleate)							
Order Tritrichomonadida (endobiotic in vertebrates (mammals, birds, reptiles, amphibia, fish))							
Tritrichomonadidae	4-5	rail-type	A-type	tube-like	present	present	*Tritrichomonas*
Simplicimonidae	4	absent	absent	tube-like	present	present	*Simplicimonas*
Monocercomonidae	4	absent	absent	cone-like	present	present	*Monocercomonas*
Dientamoebidae	0-4	absent	absent	cone-like	absent	absent	*Dientamoeba, Histomonas*
Class Trichomonadea (single karyomastigont)							
Order Trichomonadida (with costa) (endobiotic in vertebrates (mammals, birds, reptiles, amphibia) and invertebrates)							
Trichomonadidae	5-6	lamelliform	B-type	cone-like	absent	absent	*Cochlosoma, Trichomonas, Trichomitopsis, Tetratrichomonas, Pentatrichomonas*
Order Honigbergiellida (without costa) (endobiotic in vertebrates (mammals, reptiles, amphibia))							
Hexamastigidae	5-6	absent	absent	cone-like	absent	absent	*Hexamastix*
Class Hypotrichomonadea (single karyomastigont)							
Order Hypotrichomonadida (endobiotic in vertebrates (reptiles, amphibia, mammals) and invertebrates)							
Hypotrichomonidae	4	lamelliform	A-type	cone-like	present	absent	*Trichomitus, Hypotrichomonas*

*Taxa found exclusively in invertebrate hosts (such as termites and cockroaches) are not listed.

The class Trichomonadea contains a diverse range of cells with single karyomastigonts giving rise to 2-6 flagella, with one being recurrent but lacking a comb-like structure and infrakinetosomal body. Two orders are recognized: Honigbergiella (undulating membrane absent or lamelliform without supporting costa); and Trichomonadida (most with lamelliform undulating membrane supported by B-type costa and stout cone-like axostyles). The family Trichomonadida contains some 10 genera (*Cochlosoma, Lacusteria, Pentatrichomonas, Pentatrichomonoides, Pseudotrichomonas, Pseudotrypanosoma, Tetratrichomonas, Trichomitopsis, Trichomonas* and *Trichomonoides*) which vary in their cellular, organellar and flagellar configurations, zoogeography (host ranges), and biological characteristics (heterotrophs ranging from mutualists to commensals to parasites). Trichomonads (*sensu latu* = in the broadest sense) usually exhibit strong site specificity (tissue tropism) and occur in the alimentary, urogenital or respiratory tracts of their hosts, where they may cause very different types of disease. Several specialized species living in the urogenital tracts of vertebrates may cause severe inflammatory diseases, with *Trichomonas vaginalis* causing vaginitis in humans, and *Tritrichomonas foetus* causing bovine infertility. A few species living in the upper respiratory and alimentary tracts of birds may cause life-threatening diseases, including *Trichomonas gallinae* causing canker in birds. In contrast, those inhabiting the intestinal tracts of vertebrate and invertebrate hosts are often considered to be symbiotes or commensals (rather than parasites) as most infections appear benign. Rather than try to cover trichomonad biodiversity and their disparate clinical significance in one comprehensive section, it has been elected to showcase representatives in 4 separate sections, targeting:

- urogenital infections by *Trichomonas vaginalis* in humans;
- urogenital infections by *Tritrichomas foetus* in cattle;
- oral infections by *Trichomonas gallinae* in birds; and
- enteric infections by *Trichomonas, Tetratrichomonas* and *Pentatrichomonas* spp. in a wide range of hosts.

The species *Trichomonas vaginalis* infects the urogenital tracts of human hosts around the world, being found on every continent and during every season. Transmission occurs when trophozoites are transferred between males and females during coitus. Infections in males are generally asymptomatic but they act as carriers, while infections in females may cause clinical disease ranging from vaginitis to endometritis, with some linking infections to infertility and cervical cancer.

Parasite species	Size (µm)	Vertebrate Hosts	Location	Clinical signs	Distribution
Class: **Trichomonadea** (single karyomastigont) [4-6F-CLS-IKB(+CA in most genera, TA or reduced in others)]†					
Order: Trichomonadida [5-6F+LUM+BC]					
Family: Trichomonadidae [+CA]					
Genus: *Trichomonas* [5F(=4A+R)-RF]					
Trichomonas vaginalis [+AF]	4-32 x 2-15	Primates: hominid (human), plus experimental infections in cercopithecid (macaques), cebid (squirrel monkey); Rodentia: murid (mice)	urogenital system (atypically respiratory tract)	vaginitis, urethritis, endometritis	worldwide

†Coding: + = present; - = absent; #F = total number of flagella; #A = number of anterior flagella; R = recurrent flagellum; RF = recurrent flagellum extending posteriorly as free flagellum; LUM = lamelliform undulating membrane; BC = B-type costa; AF = axostyle protrudes posteriorly; CA = cone-like axostyle (*Trichomonas*-type); TA = tube-like axostyle (*Tritrichomonas*-type); CLS = comb-like structure; IKB = infrakinetosomal body.

Parasite morphology: *Trichomonas vaginalis* only forms one developmental stage, trophic forms known as trophozoites which are multi-flagellated. Under unfavourable growth conditions, these stages can round up and internalize their flagella. It has been suggested by some that these rounded stages may be ‘pseudocysts’, but most workers consider them to be degenerate forms as none have been observed to give rise to normal motile forms. Trophozoites have oval-pyriform bodies ranging from 7-32 µm in length by 5-12 µm in width depending on their state of nutrition. Those cultured in the presence of abundant food become rotund, while those cultured in less favourable conditions become thin and spatulate. Trophozoites may also appear more pleomorphic becoming amoeboid when in contact with host cells. All intact stages are uninucleate with a prominent anterior nucleus surrounded by a porous nuclear envelope. The nucleus is located just posterior or adjacent to a distinctive parabasal body formed by dictyosomes (Golgi complexes). The nucleus and parabasal body are associated with small dense basal bodies (kinetosomes) forming a single karyomastigont unit (ancestral unit with 4 kinetosomes). The kinetosomes give rise to 4 flagella which project forwards forming an anterior tuft some 8-15 µm in length. In *T. vaginalis*, a separate kinetosome gives rise to a fifth flagellum which is recurrent (directed posteriad) and attached longitudinally to the cell body forming an undulating membrane that terminates before reaching the posterior end of the cell. The undulating membrane is lamelliform in appearance (rather than rail-like) and it is underpinned by a slender elongate rod-like structure in the cell cytoplasm known as the costa, which is striated with a periodicity known as B-type (rather than A-type). Functional flagella impart motion and free cells typically exhibit a forward spiralling motion, with the undulating membrane imparting a quivering/shimmering appearance to the cell body. Trophozoites also possess a slender longitudinal hyaline rod-like structure known as an axostyle which is composed of concentric rows of microtubules forming a cone (rather than a tube). The axostyle begins near the nucleus and runs posteriorly through the cell body, sometimes protruding through the posterior end and terminating in a sharp point. Trichomonads are anaerobic and do not have mitochondria, but rather possess membrane-bound organelles known as hydrogenosomes (formerly called siderophil granules) often located in rows along the axostyle. Hydrogenosomes are energy-producing organelles that generate molecular hydrogen (by metabolizing pyruvate to acetate and carbon dioxide producing ATP by substrate-level phosphorylation with release of hydrogen ions).

Site of infection: Trophozoites of *T. vaginalis* are extracellular parasites located within the reproductive organs of humans; occurring in the urethra, vagina, cervix and uterus in women, and the urethra, prostate, seminal vesicles and epididymis of men. Trophozoites may occur free-swimming within the lumina of the tubular organs, but they may also attach temporarily to the surface of epithelial cells by small or large lateral outgrowths that are amoeboid in appearance. *T. vaginalis* is cosmopolitan in distribution and may be found in human populations throughout the world.

Pathogenesis: Infections by *Trichomonas vaginalis* usually occur in sexually active people and may result in asymptomatic carriage, especially in males, to causing acute or chronic clinical disease (trichomoniasis), with women exhibiting signs from mild vaginal discomfort and dyspareunia (painful intercourse) to severe incapacitating illness. Trophozoites inhabit reproductive organs where they may obtain nutrients for growth and survival, but they also interact with host epithelial cells producing a range of pathologies. They are strict amitochondriate anaerobes that uses hydrogenosomes to produce energy by metabolising carbohydrates or utilizing amino acids. Trophozoites thrive in acidic conditions (pH 4-6) which stimulates their growth and multiplication by longitudinal division. Parasites move using their cytoskeletal elements (microtubules, microfilaments) to interact with epithelial cells, employing a range of adhesion proteins for cell-to-cell adhesion (specific ligand-carbohydrate reactions which can also invoke parasite amoeboid transformation with the development of cellular extensions/interdigitations), cell-to-basement membrane binding (laminin-binding proteins), and extracellular matrix adhesion (fibronectin receptors). Attached parasites secrete cytotoxic enzymes (lysosomal hydrolases, cysteine proteases) which degrade host cells causing their desquamation, detachment, membrane perforation and lysis. Cellular debris is then ingested by the parasites. Feeding cells are also able to ingest resident bacteria, yeasts, and even host blood cells by phagocytosis, and they may also be ingested by host monocytes. When underlying capillary beds are exposed, trophozoites specifically target erythrocytes for iron and lipid acquisition by releasing haemolytic factors (adhesins, perforins) which facilitate erythrolysis and also direct erythrophagocytosis. Many of these molecular interactions stimulate the production of proinflammatory and chemotactic cytokines from epithelial cells and resident immune cells, including the recruitment of neutrophils which aggravate clinical signs.

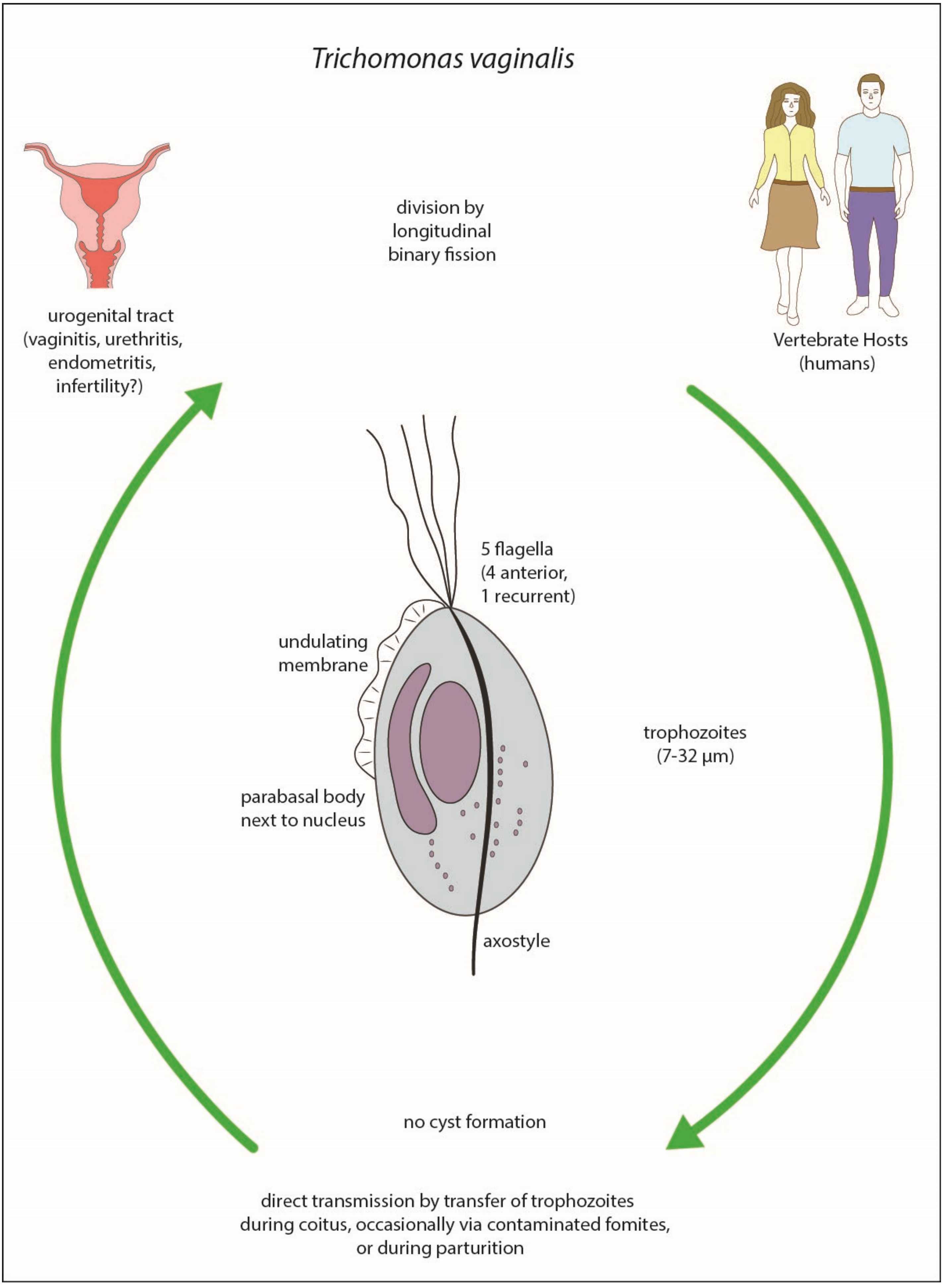
In women, *T. vaginalis* is well-adapted to the variable vaginal micro-environment with around 50% of infections being asymptomatic but the remaining 50% causing clinical disease, with cyclic symptoms and signs worsening around menses (menstruation) due to changes in host tissues (sloughing epithelia), fluids (mucus, serum), cells (microbial flora, blood cells), macromolecules (lipids, carbohydrates, proteins) and chemistry (notably pH). Menstrual flow is thought to be more conducive to nutrient and iron uptake by parasites, and trophozoites have developed efficient mechanisms to cope with oxidative stress (heat shock proteins, P-glycoproteins). Trichomoniasis is principally a disease of women in their reproductive years, with clinical manifestations rarely observed before menarche or after menopause. A wide range of symptoms have been reported, but many are commonly seen in other sexually transmitted diseases (STDs). Disease severity has been associated with differences in parasite pathogenicity (strain virulence), intensity of infection, site of infection within the urogenital system, as well as host susceptibility, hormone levels, and resident vaginal flora (esp. lactobacilli). The prepatent (incubation) period usually ranges from 4-28 days, and acute infections often subside over weeks to become chronic with the abatement of symptoms but their occasional recurrence. Once established, infections may persist in women for extended periods (weeks, months, sometimes years). Clinical infections usually cause flagrant vaginitis (significant degeneration, irritation and inflammation of the vagina) with local cellular immune responses (leucocyte infiltrations) resulting in erythema (redness), pruritus (itching), burning sensations, mucopurulent (yellow-green) frothy discharge (occasionally with blood, but leukorrhea discharge only in acute infections) and foul-smelling odours (exacerbated by the production of molecular hydrogen by parasite hydrogenosomes). The vaginal mucosa may develop a speckled appearance due to the occurrence of small punctate haemorrhagic spots, and sexual intercourse may become painful or difficult (dyspareunia). Parasites may cause irritation of the vulva resulting in erythema, oedema and diffuse vulvitis with copious leukorrhea. Infections may also extend to the cervix with women developing cervicitis, cervical erosion and sometimes colpitis macularis (strawberry cervix) formed by punctate haemorrhagic lesions (evident by colposcopy). Recent studies have also cautiously linked chronic infections to an increased incidence of cervical cancer. Parasites may continue to ascend the genital tract migrating through the cervix into the uterus causing inflammation (endometritis) with long-standing infections associated with atypical pelvic inflammatory disease and possible infertility problems (due to the generation of a uterus 'hostile' to implantation). Infections may occasionally invade the urinary system causing urethritis and cystitis with lower abdominal pain and dysuria (painful or difficult urination). Other infrequent complications include adnexitis (inflammation of uterine appendages such as fallopian tubes and ovaries), and pyosalpinx (pus-filled fallopian tubes). Women infected during pregnancy are predisposed to premature rupture of the placenta, premature labour, and low birth-weight infants. Parasites may also be transmitted to newborns as they pass through the birth canal of infected mothers, sometimes resulting in urinary tract infections in female infants and rarely pneumonia-like pulmonary infections.

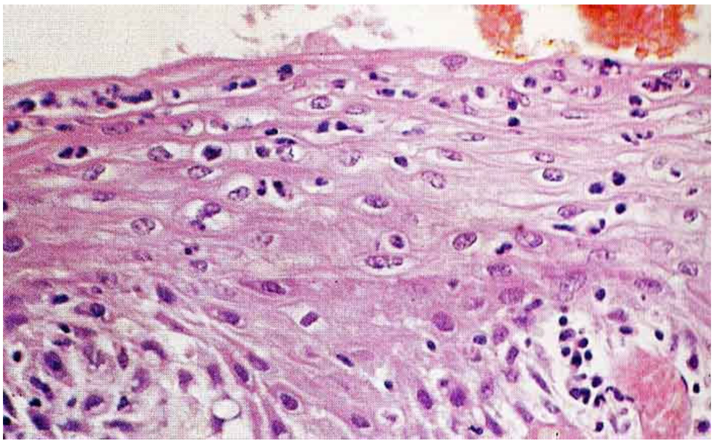
Infections in men are usually transient and do not persist for long (10 days or less). Most infections (around 90%) are asymptomatic but the men may still act as carriers. Some infections do produce mild symptomatic disease (indistinguishable from other causes of non-gonococcal urethritis) while a smaller number develop acute disease characterized by urethritis with scanty clear to mucopurulent discharge, dysuria, mild pruritus or burning sensation immediately after sexual intercourse (or urination). Infrequent complications include balanoposthitis (inflammation of the glans and foreskin), prostatitis, epididymitis, epididymo-orchitis, and possibly infertility. Although the course of infection in men is self-limiting, the parasites do not appear to elicit strong protective immune responses in their hosts. Re-infection can occur with the renewal of clinical disease, particularly in women. Immunological studies have shown that trichomonads may evade host immune responses by a number of mechanisms; including the modification of surface proteins (via antigenic shedding, antigenic variation, and antigenic shift); secreting proteins to modify host immunoglobulins (both secretory and serum antibodies) and complement (activating alternate pathways); and masking themselves with host proteins (by molecular mimicry and surface coating). Epidemiological studies have revealed risk factors for infection include sexual activity, particularly unprotected sex, promiscuity and prostitution, poor feminine hygiene, and other sexually-transmitted diseases (STDs). Indeed, *T. vaginalis* infections increase the risk of human immunodeficiency virus (HIV) transmission by causing mucosal damage and facilitating viral entry. Trichomoniasis has been reported in all racial, ethnic and socioeconomic groups around the world, with some regional geographic variation (more common in Africa). The World Health Organization considers trichomoniasis to be one of the most common STDs, with an estimated prevalence approaching 200 million and an annual incidence of several million new cases yearly.

Developmental cycle and mode of transmission: The trichomonad *T. vaginalis* has a simple direct life-cycle involving flagellated trophozoites that replicate asexually by longitudinal binary fission. The parasites have considerable proliferative potential, exhibiting exponential (binary) growth every few hours under favourable conditions. Reproduction involves the duplication of locomotory organelles and the development of 2 attractophores flanking the nucleus which subsequently become poles for division. The nuclear membrane is retained, and microtubules extend into the nucleus attaching to chromosomal centromeres. The extranuclear spindle (paradesmose) elongates and the daughter cells separate. Trophozoites reside within the urogenital tract and are unable to survive for long outside hosts. They do not form cysts, and some nonflagellated rounded forms originally thought to be 'pseudocysts' are now considered to be degenerate transient forms which cannot recover. Transmission therefore involves the direct transfer of live trophozoites between the urogenital tracts of hosts, the vast majority occurring via sexual intercourse. Occasionally, nonsexual transmission has been observed involving contaminated douche nozzles, specula or toilet seats, as well as to infants and virginal females via soiled clothing, toweling or sanitary products. Live trophozoites have been found in urine and semen after several hours of exposure to air and in swimming pool water, and trophozoites may remain viable in culture after 24 hours post-inoculation on damp fomites. Vertical transmission may also occur either during the birth process when the baby transits the birth canal, or infants become contaminated by urinary or vaginal secretions from infected women.

Differential diagnosis: The diagnosis of clinical infections by symptomatology alone is confounded by the nonspecificity of symptoms/signs as many other urogenital infections or conditions may cause similar manifestations (notably odiferous exudates). Definitive diagnosis is therefore made by the direct demonstration of motile trophozoites in vaginal, urethral or prostatic secretions by microscopy or by *in vitro* culture. Wet mounts should be examined immediately as parasites only remain motile for short periods (10 minutes) when exposed to air. Motile trophozoites move with a characteristic jerky forward rotary motion and their undulating membranes impart of shimmering appearance to the cell body. Unstained trophozoites are best observed under suboptimal illumination (condenser down or diaphragm closed to introduce diffraction) or using high contrast systems (phase-contrast or differential interference contrast, e.g. Nomarski). Alternatively, a small number of stains may be used to visualize organisms, especially Giemsa or Papanicolaou (Pap) stains, as well as a few fluorochrome stains. Fixed smears can also be examined following staining to help differentiate rounded forms of parasites from polymorphonuclear leucocytes. Suitable stains have included haematoxylin, Giemsa, acridine orange, Leishmans, periodic acid-Schiff, Fontana and Pap stains, many of which also allow gynaecological screening for cytologic abnormalities. A range of *in vitro* culture systems have been developed to amplify trichomonads from clinical samples, with broth cultures or InPouch cultures in liquid media being the most effective. Trichomonads lack the ability to synthesize many macromolecules *de novo* (particularly purines, pyrimidines and many lipids), so liquid culture media need to include all essential macromolecules, vitamins and minerals (especially serum providing lipids, amino acids, fatty acids, iron and trace metals). Suitable media include Kupferberg medium, Diamond's TYM medium (trypticase-yeast-maltose), Diamond's TYI-S-33 medium (trypticase-yeast-iron with foetal calf serum and vitamin 107-Tween 80 mix), Trichosel medium, Linstead's semi-defined medium (modified CMRL 1066 medium) and Linstead's chemically defined media (DL7, DL8). Culture is often laborious and tedious, with results only becoming available after 2-7 days. Studies have shown that trophozoites can be grown on various cell lines (e.g. McCoy cells) in serum-free tissue culture, but such processes are not routinely available due to cost. Some studies, however, indicated that tissue culture could be used to examine parasite virulence factors, such as haemolytic activity, adherence and cytotoxicity. Experimental animal models have been used for *in vivo* culture in monkeys, hamsters, guinea pigs, rats, mice, cattle, and dogs, but most were poor models which did not mimic disease. Nevertheless, some promising results were obtained in squirrel monkeys (sustained symptomatic disease and horizontal transmission, but some confusion with indigenous trichomonads) and mice (intravaginal inoculation producing disease but usually requiring pre-oestrogenization, intraperitoneal inoculation linked to virulence as evident by visceral organ necrosis, and subcutaneous inoculation linked to virulence as evident by abscess formation). Various immunological techniques have been used to detect specific host antibodies to trichomonad antigens, including gel diffusion, complement fixation, agglutination, fluorescence and enzyme immunoassays which varied in both sensitivity and specificity by reacting with variety of antigenic markers. Monoclonal antibodies have also been developed and used in agglutination, fluorescence and enzyme immunoassays to detect parasite antigens in clinical samples. More recently, molecular biological techniques have been used to characterize parasites in clinical samples following the amplification of nuclear gene sequences (18S ribosomal RNA, ferrodoxin, beta-tubulin, adhesion protein genes, and a highly-repetitive 2-kb DNA sequence) by conventional, nested and real-time polymerase chain reaction (PCR) techniques, and dot-blot DNA hybridization assays using radioactive isotopes or fluorochrome labels.

Treatment and control: Clinical infections may be treated using drugs effective against anaerobic bacteria and protozoa, notably the nitroimidazole drug (metronidazole) and derivatives (tinidazole, ornidazole, secnidazole, flunidazole, nimorazole and carnidazole). Some trichomonacidal activity has also be found using aromatic polyenes (hamycin), some antibiotics (paromomycin, anisomycin, purpuromycin), nitrothiazole derivatives (niridazole), nitrothiazolyls (nitazoxanide), nitrofurans (furazolidone, nifaratel), a microtubule-inhibitor (mebendazole) and some salt solutions (sodium nitrite, sodium nitroprusside, Roussin's black salt), but their activity was variable and many exhibited adverse side-effects. Nitroimidazoles are usually given orally, although some formulations are available as topical vaginal medications (creams and gels) or pessaries. Metronidazole is well tolerated despite its metallic taste, but alcohol consumption is contraindicated due to antabuse-like side-effects (ethanol sensitivity) and other adverse reactions (nausea, vomiting, headache, insomnia, dizziness, drowsiness, rash). In addition, metronidazole should not be administered to pregnant women in the first trimester as it may cross the placenta causing problems. Instead, pregnant women with severe symptoms are best treated with intravaginal pessaries containing clotrimazole, povidone-iodine, nonoxynol-9 or arsenical compounds. The treatment of disease in neonates (babies infected during birth) is often curtailed as most infections resolve after 3-6 weeks as maternal oestrogen levels wane. Regrettably, there are growing numbers of treatment failures (4-10%), either due to non-compliance or reinfection, but more usually due to the emergence of drug-resistant strains of parasites. Metronidazole is a pro-drug which is reduced by the enzyme pyruvate: ferredoxin oxidoreductase (PFOR) to yield cytotoxic nitro radical anions that interfere with parasite DNA synthesis. Resistance to metronidazole may be aerobic involving oxygen-scavenging pathways and ferredoxin or anaerobic involving the reduction or cessation of PFOR and hydrogenase activity. A range of preventive measures have been adopted to reduce the transmission of infections, including regular health screening and treatment of patients and sexual partners, practicing safe sex (using condoms), abstinence or monogamy, maintaining high standards of feminine hygiene, and using suppositories and alkaline douches to lower vaginal pH and reduce symptoms. Many countries have instigated public awareness and health education campaigns targeting sexually-transmitted diseases. The prospects for vaccine appear to be poor because natural infections do not induce long-term immune protection despite the demonstration of specific antibodies (serum and mucosal) and some local inflammatory responses. Both humoral and cellular responses rapidly wane (within 6 months), particularly after treatment, and hosts are susceptible to re-infection and disease. The parasites have also been shown to have adopted many different immuno-evasion tactics, involving antigenic variation, molecular mimicry and coating themselves in host molecules. Nevertheless, several vaccine trials were conducted with mixed results. Patients with refractory infections that were inoculated with heat-inactivated trophozoites exhibited some clinical improvement, as did those inoculated with heat-inactivated 'abnormal strains' of lactobacilli isolated from clinical cases. The apparent cross-protection afforded by the latter studies was thought to be due to microbial interactions favouring resident flora that were inhibitory to parasite growth. Experiments on a mouse model found that subcutaneous injections of whole trophozoites with and without adjuvants prior to their oestrogenization and prebiotic vaginal inoculation with lactobacilli led to the resolution of some infections and the development of some protection in vaccinated mice, suggesting the trichomonad-bacterial interactions could be further exploited for therapeutic and prophylactic purposes.


Trichomonas vaginalis
urogenital tract
(vaginitis, urethritis,
endometritis,
infertility?)
division by
longitudinal
binary fission
Vertebrate Hosts
(humans)
5 flagella
(4 anterior,
1 recurrent)
undulating
membrane
parabasal body
next to nucleus
trophozoites
(7-32 µm)
axostyle
no cyst formation
direct transmission by transfer of trophozoites
during coitus, occasionally via contaminated fomites,
or during parturition

	
Trichomonas vaginalis trophozoites	*Trichomonas vaginalis* vaginitis