Tritrichomonas foetus
(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. Recent studies have reassigned *Tritrichomonas* spp. to a novel class based on ultrastructural features and genotypic differences. The species *Tritrichomonas foetus* has variously been associated with bovine abortion and infertility, chronic diarrhoea in cats and subclinical infections in pigs.

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:	Tritrichomonadea (single mastigont, comb-like structure, infrakinetosomal body)
Order:	Tritrichomonadida (variable possession of undulating membrane and costa)
Family:	Tritrichomonadidae (4-5 flagella, rail-type undulating membrane, A-type costa, tube-like axostyle)
Genus:	*Tritrichomonas* (parasites/commensals in tubular organs of vertebrates)
Species:	*T. foetus* (causes abortion and infertility in cattle)

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.

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

The genus *Tritrichomonas* was separated from other trichomonads initially on the basis of ultrastructural studies, notably involving the possession of different undulating membranes (rail-type), costae (A-type), axostyles (tube-like) and the presence of comb-like structures and infrakinetosomal bodies. Molecular characterization studies, however, revealed that many of these characters were not apomorphic but plesiomorphic, with many related taxa varying considerably in their structural complexity, and often demonstrating reductive evolution by lacking (or having lost) various features. The novel class Tritrichomonadea was erected to accommodate a diverse range of cells with 1-2 nuclei and 0-5 flagella but otherwise varying in their possession of various cytoskeletal, membranous and cytoplasmic elements. The order Tritrichomonadida contains 4 families: one family with 'trichomonad' features (Tritrichomonadidae (genus *Tritrichomonas*) with uninucleate cells, 4-5 flagella, rail-type undulating membranes, A-type costae, tube-like axostyles); and 3 families with greatly simplified structures (Dientamoebidae syn. Protrichomonadinae (4 genera: *Dientamoeba, Histomonas, Parahistomonas, Protrichomonas*) with uni- to bi-nucleate cells, 0-4 flagella, cone-like axostyles (if present), but lacking undulating membranes, costae, comb-like structures and infrakinetosomal bodies; Monocercomonadidae (one genus *Monocercomonas*) with uninucleate cells, 4 flagella, marginal plates, cone-like axostyles, but lacking undulating membranes and costae; and Simplicimonadidae (one genus *Simplicimonas*) with uninucleate cells, 4 flagella, marginal plates, tube-like axostyles, but lacking undulating membranes and costae). Most cognate species have been recorded as symbiotes (mutualists, commensals or parasites) of insects, but a few species infect vertebrate hosts, including humans and domestic animals.

Around 16 *Tritrichomonas* spp. have been found in the alimentary tracts of their mammalian, avian or amphibian hosts, but one species, *Tritrichomonas foetus,* also occurs in the urogenital tract of cattle. Infections by *Tritrichomonas foetus* are of considerable veterinary importance as they are responsible for serious economic losses in many cattle breeding areas throughout the world by causing reproductive failure. However, recent molecular phylogenetic studies have cast doubt on the host specificity, site of infection and pathogenicity of *Tritrichomonas foetus*. Genetic characterization studies have detected almost identical genotypes in other tissues and organs of non-bovine hosts: notably, the digestive tracts of diarrhoeic cats and dogs and asymptomatic pigs. Isolates identified as *Tritrichomonas suis* from the digestive tracts of pigs have been shown to be genetically similar to *Tritrichomonas foetus* isolates from the urogenital tracts of cattle by random amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) analyses as well as by sequencing at 11 loci covering ribosomal RNA (rRNA), including internal transcribed spacer regions 1 and 2, cysteine proteases (CP) 1, 2, 4, 5, 6, 7 and 9 and malate dehydrogenase 1, although small differences were evident at two other loci (CP8 and elongation factor 1-alpha (EF-1α)). On the basis of their genetic similarities, many authors now consider these species to be synonymous. In addition, other studies have shown isolates from diarrhoeic cats to be genetically similar to *Tritrichomonas foetus* isolates from cattle at 11 loci covering internal transcribed spacer region 2 of rRNA, EF-1α, CP 1, 2, 4, 5, 6, 7, 8 and 9, and malate dehydrogenase 1 (MDH1). It was initially suggested the cat isolates be named *Tritrichomonas blagburni* but some consider there were not enough differences for speciation from *Tritrichomonas foetus*. Information on the resultant *Tritrichomonas foetus* complex has therefore been partitioned into three groups corresponding to symptomatic urogenital infections in cattle, symptomatic intestinal infections in cats and asymptomatic intestinal infections in pigs.

Parasite species†	**Size (µm)**	**Vertebrate Hosts**	**Location**	**Clinical signs**	**Distribution**
Class: **Tritrichomonadea** (uninucleate to binucleate) [0-5F±RUM+AC+CLS+IKB+CA/TA]					
Order: Tritrichomonadida (as for class)					
Family: Tritrichomonadidae (uninucleate, suprakinetosomal body present) [4-5F+TA] (1 genus)					
Tritrichomonas [4F(=3A+R)+RF+AC]					
Tritrichomonas foetus (syn. *Trichomonas foetus*) complex					
Tritrichomonas foetus (cattle isolates) [+AF]	8-25 x 3-15	Artiodactyla: bovid (cattle), rarely cervid (roe deer), camelid (camel); Perissodactyla: equid (horse), plus experimental infection in Rodentia: murid (mice, rats), caviid (guinea pigs); Lagomorpha: leporid (rabbit); Carnivora: canid (dog), felid (cat); Artiodactyla: bovid (goat, sheep), suid (pig): Primates: cercopithecid (macaques); Aves: Galliformes (chickens, turkeys); atypical infection in Primates: hominid (human)	uterus, prepuce (atypically human respiratory tract)	vaginitis, abortion, infertility	worldwide
Tritrichomonas foetus (syn. *Tritrichomonas blagburni*) (cat isolates) [+AF]	10-25 x 3-15	Carnivora: felid (cat), canid (dog), plus paratenic transport in Mollusca: gastropod (slugs)	ileum, caecum, colon	diarrhoea	Europe, North America, Australia, Asia

Tritrichomonas foetus (syn. *Tritrichomonas suis, Trichomonas suis*) (pig isolates) [+AF]	9-16 x 2-6	Artiodactyla: suid (pig), plus experimental infection in bovid (cattle)	nasal cavity, stomach, caecum, colon	non-pathogenic (but has induced abortion experimentally in pigs)	worldwide
other *Tritrichomonas* species					
Tritrichomonas augusta (syn. *Trichomonas*)		Urodela: cryptobranchid (hell-bender), salamandrid (giant newt, common newt, spotted salamander), ambystomid (marbled salamander, tiger salamander), plethodontid (red-backed salamander, slimy salamander, dusky salamander); Anura: bufonid (Fowler's toad), pelobatid (spadefoot frog), ranid (bullfrog, southern leopard frog, leopard frog, yellow-legged frog, Pacific coast frog), hylid (tree frog, swamp tree frog)	intestines		worldwide
Tritrichomonas beckeri	4-8 x 2-5	Cuculiformes: cuculid (yellow-billed cuckoo)	large intestines	non-pathogenic	Americas
Tritrichomonas caviae (syn. *Trichomonas*)	10-22	Rodentia: caviid (guinea pig)	caecum, colon	non-pathogenic	worldwide
Tritrichomonas eberthi (syn. *Trichomonas eberthi*) [+AF]	8-14 x 4-7	Galliformes: phasianid (chickens, turkeys); Anseriformes: anatid (ducks)	caecum	non-pathogenic	worldwide
Tritrichomonas enteris	6-12 x 5-6	Artiodactyla: bovid (cattle)	colon	non-pathogenic	Europe, India
Tritrichomonas equi (syn. *Trichomonas faecalis, equi*)	10-11 x 5-6	Perissodactyla: equid (horse, donkey)	caecum, colon	diarrhoea, colitis	worldwide
Tritrichomonas floridanae (syn. *Trichomonas*)	7-12 x 3-7	Galliformes: tetraonid (partridge), phasianid (valley quail, bobwhite quail, California quail)	caecum	non-pathogenic	North America
Tritrichomonas gigantica	7-22 x 3-10	Galliformes: phasianid (quail)	caecum	non-pathogenic	nr
Tritrichomonas indica [*nomen nudum*]	9-18 x 5-14	Galliformes: phasianid (black-breasted quail)	caecum	non-pathogenic	India
Tritrichomonas minuta (syn. *Trichomonas*)	4-9	Rodentia: murid (rats, mice), cricetid (hamster)	large intestines	non-pathogenic	worldwide
Tritrichomonas mobilensis	7-11 x 1-3	Primates: cebid (squirrel monkey)	large intestines	non-pathogenic	widespread in laboratory colonies
Tritrichomonas muris (syn. *Trichomonas criceti*)	12-20	Rodentia: murid (rats, mice), cricetid (voles, hamster)	large intestines	non-pathogenic	worldwide
Tritrichomonas nonconforma		Sauria: dactyloid (anoles)	cloaca		Cuba
Tritrichomonas pondicerianae [*nomen nudum*]	9-17 x 8-13	Galliformes: phasianid (grey partridge)	caecum	non-pathogenic	India
Tritrichomonas rotunda		Artiodactyla: suid (pig)	large intestines	non-pathogenic	Europe
Tritrichomonas simhii		Anura: ranid (pond frog)			India

†Coding: + = present; - = absent; #F = total number of flagella; #A = number of anterior flagella; R = recurrent flagellum; RF = recurrent flagellum extending posteriorly as free flagellum; RUM = rail-type undulating membrane; AC = A-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: *Tritrichomonas foetus* forms motile trophic developmental stages known as trophozoites which are multi-flagellated. The parasites do not form true encapsulated cysts, but under unfavourable growth conditions, some cells can round up and lose their flagella. It has been suggested that these 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 spindle-pyriform bodies ranging from 8-25 μm in length by 3-15 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. All intact stages are uninucleate with a prominent anterior ovoid nucleus located adjacent to a distinctive sausage-shaped 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 3 flagella which project forwards forming an anterior tuft some 8-15 μm in length. In *Tritrichomonas foetus*, the fourth kinetosome gives rise to a recurrent flagellum (directed posteriad) that is attached longitudinally to the cell body forming an undulating membrane. The recurrent flagellum runs the whole length of the body and extends beyond the posterior margin as a free flagellum. The undulating membrane is rail-like in appearance (rather than lamelliform) 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 A-type (rather than B-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 tube (rather than a cone). The axostyle begins near the nucleus and runs posteriorly through the cell body, protruding through the posterior end and terminating in a sharp point. Tritrichomonads also possess a unique comb-like structure and infrakinetosomal body (both structures lacking in typical trichomonads). Tritrichomonads (like all trichomonads) are anaerobic and do not have mitochondria, but rather possess membrane-bound organelles known as hydrogenosomes (formerly called siderophil granules) that are located along the axostyle, appearing as a 'chromatic ring' under the light microscope and as osmiophilic granules under the transmission electron microscope. 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: *Tritrichomonas foetus* has been recorded primarily in the urogenital tract of cattle, but may also occur in the alimentary tracts of cats and pigs. In cattle, trophozoites are usually found in cows in the vagina, although they may colonize the surface of the entire genital system (vagina, cervix, endometrium, oviducts) and even migrate to the uterus. Parasites may be found in the preputial cavity of bulls on the surface of the stratified squamous epithelium of the penis and proximal foreskin, although some infections may extend to the seminal vesicles, epididymis and testes. In cats, trophozoites occur in the mucosal lining of the ileum, caecum, colon and rectum (especially on the surface of the colon and in colonic crypts), and rarely in the urogenital tract. In pigs, parasites have been detected in the nasal cavities, stomach and intestines. Other *Tritrichomonas* spp. have been described from the alimentary tracts of rodents (rats, mice, hamsters, guinea pigs, voles), equids (horse, donkey), primates (squirrel monkey) as well as some birds (cuckoos, chickens, turkeys, partridge, quail, ducks), lizards (anoles), and amphibians (newts, salamanders, toads and frogs).

Pathogenesis: Infections by members of the *Tritrichomonas foetus* complex have been associated with symptomatic urogenital infections in cattle, symptomatic intestinal infections in cats and asymptomatic intestinal infections in pigs (infections in different host species are thought to be due to different parasite genotypes). Trophozoites lack cytostomes but capture food through the cell surface by pinocytosis and phagocytosis resulting in the accumulation of cytoplasmic food vacuoles of variable sizes. The parasites feed on solutes and particulate material (including bacteria) within the lumina of tubular organs or in contact with mucosal surfaces. While light infections may remain asymptomatic or subclinical, the presence of growing numbers of parasites may cause significant tissue irritation and inflammation with attendant pathologies.

Infections in cattle by *Tritrichomonas foetus* s.s. are of considerable veterinary importance as they cause bovine tritrichomoniasis, a sexually transmitted disease, resulting in serious economic losses mainly in extensively-managed herds due to reproductive failure (abortion, endometritis and infertility). Cows recently infected may develop mild vaginitis, cervicitis or endometritis, with few overt clinical signs other than mucopurulent vaginal discharges. Most infections are self-limiting and parasites are cleared from the vagina and uterus of non-pregnant heifers after 6-16 weeks, but hosts do not develop any long-lasting immunity. In pregnant cows, infections do not interfere with fertilization, implantation or early foetal development, but they can result in foetuses being lost early in gestation with resorption or abortion (typically around 2-3 months, rarely 4). Abortions may be missed by producers who think that the cows simply had not conceived. Most cows recover but some may retain foetal material which becomes macerated or mummified leading to pyometra, chronic endometritis and temporary infertility. A few cows may remain infected throughout pregnancy and into the following breeding season, but such carrier cows are rare. Disease is typically associated with lowered fertility in infected herds, with poor pregnancy rates, many services per conception, long calving intervals, extended calving periods, calf crop reduction, and wide variation in weaning weights. The severity of disease has been found to depend on differences in parasite virulence (strain variation), the intensity of infection, the mechanical action of parasites on mucosal surfaces, their feeding activities involving the secretion of enzymes, pinocytosis and phagocytosis, host susceptibility and the extent of changes to the intrauterine environment. Infections in bulls usually do not cause any clinical signs of disease, although some animals develop inflammation of the penis (balanitis) with small nodules and preputial discharges. Infections often become

chronic and bulls may act as asymptomatic carriers for years. This presents a problem for artificial insemination programs, because most techniques used to cryopreserve flagellated sperm also cryopreserve flagellated trophozoites. Risk factors for infection include age (infections accumulate in bulls > 4 years old), herd size (higher prevalence in larger herds), high bull:cow ratios (more sexual contacts), multiple bulls in service (more cross-infections), cohort co-mingling (more transmission) and breed susceptibility (higher prevalence in *Bos taurus* (esp. Angus, Charolais, Hereford and Simmental breeds) than in *Bos indicus* or hybrids).

Infections in cats by *Tritrichomonas foetus* s.l. (syn. *T. blagburni*) have been associated with lymphocytic and plasmacytic inflammation of the colon resulting in colitis (large bowel diarrhoea) with increased frequency of defaecation of semi-formed to liquid faeces sometimes containing fresh blood or mucus. Faeces are often yellow-green in colour, gassy and malodourous. The diarrhoea may be chronic or intermittent and may range from mild to intractable. Animals may also exhibit faecal incontinence, tenesmus, flatulence, abdominal pain, anal irritation, and sometimes systemic signs such as anorexia, vomiting, depression and weight loss. The course of infection waxes and wanes over several weeks but may persist for 5-24 months. Most cats go into clinical remission but still act as asymptomatic carriers, while some may relapse for short periods with worse diarrhoea. Risk factors include age (mainly a disease of kittens and young cats < 12 months of age), breed (pedigree cats, particularly Siamese and Bengal), population density (catteries, cat shelters, breeding centres), attendance at cat shows, and concurrent enteric infections. Infections in pigs by *Tritrichomonas foetus* s.l. (syn. *T. suis*) are asymptomatic with the organisms essentially considered to be harmless endocommensals.

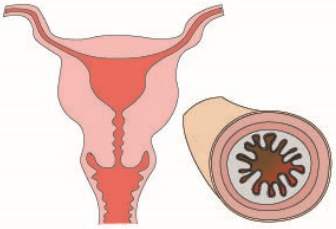
Developmental cycle and mode of transmission: Tritrichomonads have simple life-cycles involving the asexual multiplication of trophozoites in host organs and their transmission to new susceptible hosts by direct contact. They do not form environmentally-resistant cysts and the trophozoites do not survive for long outside of hosts in aerobic conditions. The trophozoites of some species do round up and lose their flagella forming stages known as pseudocysts, but it has not been established whether they are resistant or degenerate forms. Trophozoites multiply by longitudinal binary fission in which the nuclear membrane persists during division (type of mitosis known as cryptopleuromitosis). Parasites may rapidly form large populations by exponential (binary) growth when conditions are optimal. Infections in cattle are transmitted sexually mainly by natural mating (coitus), but sometimes mechanically during artificial insemination or vaginal examination. While parasites may survive semen cryopreservation (especially if samples are contaminated with preputial fluids), transmission via artificial insemination is rare. Bulls are the primary reservoirs for infection and may be long-term (even life-long) carriers. Cows become immune to re-infection, at least for the breeding season in which they were infected. Infections in cats are spread by direct contact (licking, grooming, nursing) and by faecal-oral transmission when shared litterboxes allow parasites from faeces to be transferred to the paws and fur of other cats to be ingested during grooming. Trophozoites have been found to live in wet cat faeces for 3 days and in contaminated cat food for 5 days. Experimental studies have also shown that terrestrial slugs (leopard and yellow cellar slugs) fed on spiked cat food shed viable trophozoites in their faeces and thus may act as paratenic hosts for the dispersal of cat isolates. The prepatent period for infections in cats ranged from 2-7 days and infections were found to persist in cats for up to 200 days. Infections in pigs are thought to be transmitted through direct contact as well as the faecal-oral contamination of fomites.

Differential diagnosis: Tritrichomoniasis may be indicated as a cause of reproductive failure in cattle based on clinical history, signs of early abortion, and apparent infertility with repeated returns to service or irregular oestrous cycles. Infections are then confirmed by the detection of trophozoites in clinical samples by microscopy, culture or molecular techniques. Motile organisms may be detected in preputial smegma, cervico-vaginal mucus, pyometra discharges, uterine washings, placental fluid, and sometimes from stomach contents of aborted foetuses. Samples may be collected using swabs, brushes, scrapers, insemination pipettes, straws, gauze sponges or aspiration syringes, and dilute samples may be concentrated by centrifugation. Parasite numbers fluctuate in cows during their oestrous cycles with higher numbers found a few days before oestrus. Samples from cats best include freshly-voided or manually-extracted faeces, rectal swabs, or colon flushes. Many routine microbiological techniques (such as fixed smears, coprological sedimentation or floatation) destroy the fragile trophozoites, so it is best to prepare fresh wet smears for high-contrast brightfield, phase-contrast or interference-contrast microscopy. Motile parasites may be identified by their characteristic patterns of motility, involving jerky tumbling motions of cells that appear to shimmer due to rapid movements of their undulating membranes. If required, smears can be stained with Giemsa, silver, iron haematoxylin, malachite green, methylene blue, or acridine orange, but fixation using chemicals, heat or desiccation usually causes cells to contract or lyse. Post-mortem tissue samples can be processed for histological examination following staining with haematoxylin and eosin, Giemsa or periodic acid-Schiff-reagents. Trophozoites may be cultured *in vitro* in semi-defined liquid media (Diamond's media, modified Diamond's media, or InPouch TF selective media) for several days at 25-37°C (trophozoites require added nutrients as they cannot synthesize some macromolecules de novo). Parasites have also been grown on various tissue culture cell lines where they exhibit some cytotoxicity (highly toxic to human cervical (HeLa) cells, bovine lymphosarcoma (BL-3) cells, moderate toxicity to Vero cells, African green monkey kidney cells, and apoptotic to bovine vaginal epithelial (BVEC) cells, and porcine intestinal epithelial (IPEC) cells). Several immunoserological tests (agglutination, haemolytic and enzyme immunoassays) have been developed to detect host antibodies in serum or cervicovaginal mucus, but they generally only detected transient responses. Monoclonal antibodies were also produced and used for the immunohistochemical localization of parasites in host tissues. A murine intradermal test was used to test for virulence by measuring skin lesions after the inoculation of known numbers of fixed cultured trophozoites. More recently, modern molecular biological techniques have been applied to the detection and characterization of parasite isolates by restriction fragment length

polymorphism analyses, random amplified polymorphic DNA analyses, *in situ* hybridization (ISH) using fluorochrome- or chromogenic-probes, and direct gene sequencing following the polymerase chain reaction (PCR) amplification of various gene sequences (including small and large subunit ribosomal RNA and internal transcribed spacers 1 and 2, cysteine proteases (CP) 1, 2, 4, 5, 6, 7, 8, 9, cytosolic malate dehydrogenase (MDH1), and elongation factor 1-alpha (EF-1α)). Different genotypes of *Tritrichomonas foetus* have been detected in cattle, cats and swine.

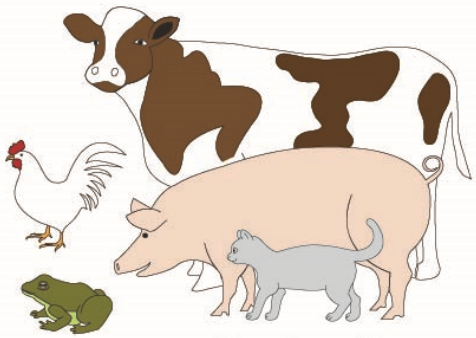
Treatment and control: Options for the treatment and prevention of tritrichomoniasis depend largely on the types and numbers of hosts to be treated. Chemotherapy is rarely attempted in cattle as it is tedious, expensive and only partially effective. Systemic treatments with nitroimidazoles (metronidazole, dimetridazole) often produce adverse side-effects and have led to the emergence of drug-resistant parasite populations. Preputial infections in bulls may respond to treatment with imidazole (ipronidazole) salves or ointments, but many persistent infections become entrenched in the inner genital tubes and testes making them refractory to treatment. Effective control depends on management strategies to detect and cull bulls and cows before their introduction into herds or breeding. Infected animals may be identified by periodic testing and all new and replacement livestock should be screened whilst in quarantine isolation. It is often recommended that bulls be replaced after 4 seasons as younger bulls are better able to eradicate infections whereas they accumulate in older animals. Artificial insemination programs may be used to avoid infections, but only when donor animals are thoroughly screened. Pregnancy testing should be used to identify fertile cows as soon as practicable and infertile animals should be investigated before returned to service. Some producers reduce their breeding seasons to < 4 months for logistic reasons. Good husbandry includes proper nutrition and sanitation as well as avoiding stressful conditions (such as over-crowding, co-mingling herds, and concomitant infections). Several studies have developed experimental vaccines using whole parasites and purified extracts to help eliminate infections before foetal loss, but without necessarily avoiding parasite colonization in the first 40 days. Some vaccines have shown promise, particularly those stimulating strong mucosal responses involving secretory antibodies (IgA, IgG1) and milder systemic responses (IgG2, IgG1). Infections in cats have responded well to treatment with nitroimidazoles (metronidazole, tinidazole, ronidazole), aminoglycosides (paromomycin), benzimidazoles (fenbendazole), nitrofurans (furazolidone), and thiazolides (nitazoxanide), but with some adverse side effects including neurological toxicity. It is recommended that all cats in contact be treated to help eradicate infections in catteries. The adoption of preventive measures designed to reduce transmission should include separating cohorts, limiting contact between cats, strict hygiene and sanitation, disinfecting fomites, equipment, and feeding containers, excluding invertebrates (e.g. slugs and snails) and providing clean food and water. Infections in pigs are asymptomatic and are not treated.

Tritrichomonas

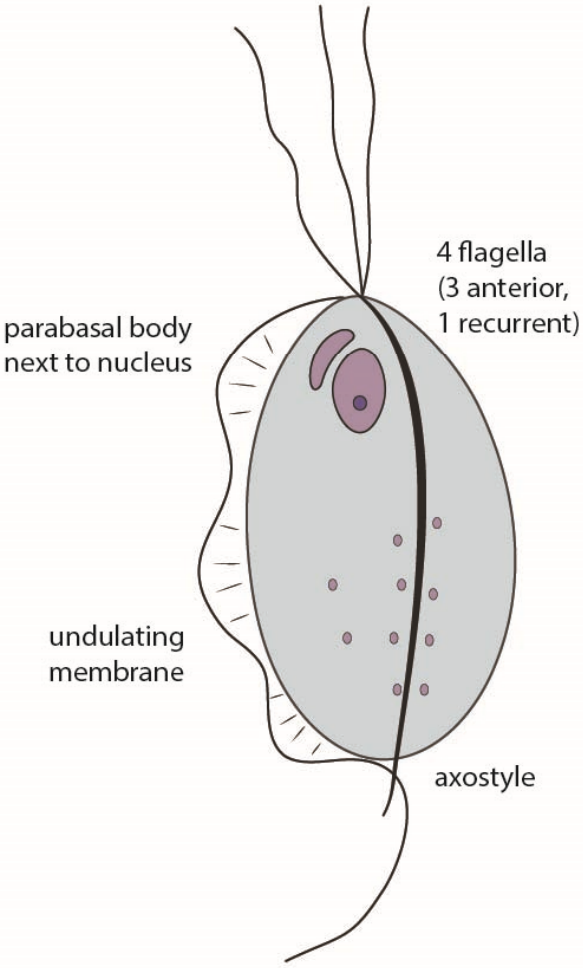


urogenital tract, gut
(vaginitis, endometritis,
abortion, infertility,
rarely diarrhoea)

division by
longitudinal
binary fission



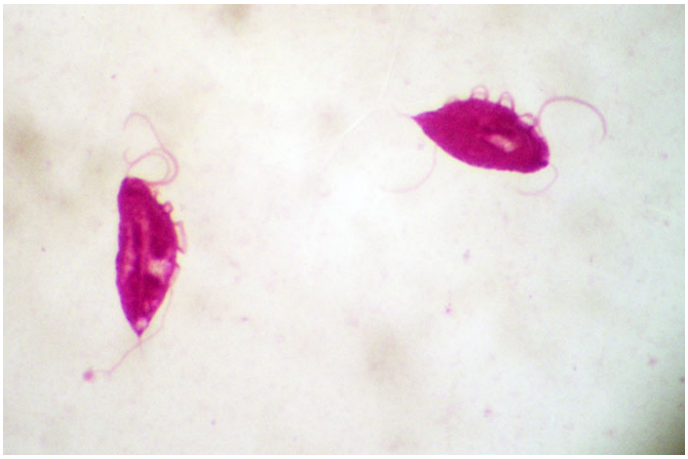
Vertebrate Hosts
(mammals, birds,
amphibians)



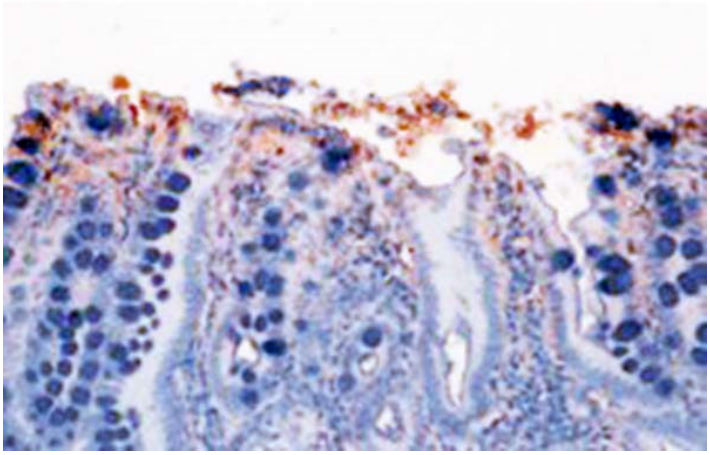
trophozoites
(8-25 µm)

no cyst formation

direct transmission by transfer of trophozoites
by close contact (coitus, parturition, parental care)
or via contaminated fomites



Tritrichomonas foetus trophozoites



Tritrichomonas foetus uterine inflammation