Histomonas
(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. Dientamoebids, however, do not have recurrent flagella or associated structures (histomonads have only one flagellum and *Dientamoeba* have none). Most are parasites of insects although a few species infect domestic animals. They do not form cysts so horizontal transmission involves direct contact, and sometimes other hosts. Histomonads parasitize galliform birds and use caecal nematodes as intermediate hosts and earthworms as paratenic hosts. One species (*H. meleagridis*) causes severe disease in turkey poults when aflagellate amoeboid stages invade host tissues.

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:	Tritrichominadida (variable possession of undulating membrane and costa)
Family:	Dientamoebidae (0-4 flagella, undulating membrane absent, costa absent, cone-like axostyle, comb-like structure absent, infrakinetosomal body absent)
Genus:	*Histomonas* (parasitic in caeca/liver of birds)
Species:	*H. meleagridis* (causes histomoniasis (infectious enterohepatitis or blackhead disease) in turkeys)

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).

Family	Key characters to 'trichomonad' families in vertebrates*						Representative genera
	Number of flagella	Undulating membrane	Costa	Axostyle	Comb-like structure	Infra-kinetosomal body	
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 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 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 only in invertebrate hosts (such as termites and cockroaches) are not listed

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.

The genus *Histomonas* contains 2 species which form uninucleate cells within the caeca of avian hosts, mostly galliforms. One species (*H. meleagridis*) forms flagellated lumen forms (trophozoites with one flagellum) as well as non-flagellated tissue forms (rounded trophozoites). Transmission between birds occurs when tissue forms infect caecal nematodes as paratenic hosts and are transferred to new hosts within nematode eggs. The species *Histomonas meleagridis* causes severe disease in birds, sometimes known as blackhead. Infections are cosmopolitan and prevalent in gallinaceous fowl, esp. young turkeys (up to 14 weeks of age) and chickens (up to 16 weeks of age). Infections also occur in peafowl, grouse and pheasant, but reports in non-galliform birds, such as ostriches, are rare and ducks and geese have proven refractory to experimental infection. The other species (*H. wenrichi*) only forms flagellated lumen forms (trophozoites with 4 flagella), and transmission is thought to involve contamination of food and water.

Parasite species	Length (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: Dientamoebidae (uninucleate to binucleate, marginal plate present) [0-4F-UM-C±CA-CLS-IKB]					
Histomonas (flagellated lumen form, sometimes aflagellar tissue form, caecal nematode vector) [0-4F]					
Histomonas meleagridis (syn. *Amoeba*) [0-2F]	6-20	Galliformes: phasianid (turkeys, chickens, chukar, pheasant, quail, grouse, jungle fowl, guinea fowl, peafowl), occasionally Struthioniformes: struthionid (ostriches) [plus invertebrate vector Nematoda: ascaridid (caecal worm *Heterakis gallinarum*), the eggs of which may also be ingested by paratenic hosts: Clitellata: lumbricid (earthworms); Diptera: muscid (houseflies)]	caeca (spreading to liver, spleen, kidney, lungs)	anorexia, diarrhoea, depression, ruffled feathers, blackened skin around head (blackhead) (or chronic wasting disease)	worldwide
Histomonas wenrichi (syn. *Parahistomonas*) [4F]	9-30	Galliformes: phasianid (turkeys, chickens, pheasant)	caeca	non-pathogenic	

†Coding: + = present; - = absent; #F = total number of flagella; UM = undulating membrane; RUM = rail-type undulating membrane; C = costa; AC = A-type costa; CA = cone-like axostyle (*Trichomonas*-type); TA = tube-like axostyle (*Tritrichomonas*-type); CLS = comb-like structure; IKB = infrakinetosomal body.

Parasite morphology: Histomonads form 2 different types of developmental stages: flagellated trophozoites and non-flagellated amoeboid forms (sometimes with small but distinct lobose pseudopodia). All stages are uninucleate and pleomorphic in shape (often rounded, sometimes spindle-shaped) ranging in size from 5-30 μm for trophozoites and 8-19 μm for amoeboid forms. The nucleus is ovoid and located eccentrically adjacent to a V-shaped parabasal body formed by dictyosomes (Golgi complexes) and flagellar basal bodies (kinetosomes). The kinetosomes give rise to eukaryotic flagella (with 2 central microtubules and 9 peripheral doublets) which extend from the anterior margin of the cells for 2-8 μm. *H. meleagridis* trophozoites possess one short flagellum, while those of *H. wenrichi* have 4 longer flagella. Trophozoites also possess a longitudinal rod-like structure called an axostyle that is composed of concentric rows of microtubules forming a cone (rather than a tube). Histomonads 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. 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). Histomonads do not form true encapsulated cysts, but some cells round up and lose their flagella apparently forming small so-called 'resistant' stages 4-11 μm in diameter with an eosinophilic cytoplasm.

Site of infection: Infections are found in galliform birds (turkeys, chickens, chukar, pheasant, quail, grouse, jungle fowl, guinea fowl, peafowl), and occasionally in struthioniform birds (ostriches). Trophozoites infect the caecum while invasive forms can penetrate the epithelium and be disseminated to the liver and other organs (e.g. spleen, kidney, lungs). Parasites may also be taken up by caecal nematodes (*Heterakis gallinarum*) where they infect the ovaries and then worm eggs. Infected worm eggs may be ingested and transported by invertebrate paratenic hosts, including earthworms and houseflies.

Pathogenesis: Infections in birds may be asymptomatic, subclinical or cause severe clinical disease known as histomoniasis, infectious enterohepatitis, necrotizing typhlohepatitis or 'blackhead', especially in turkey poults. The severity of disease varies according to differences in host susceptibility, intensity of infection, parasite virulence, frequency of exposure, and concomitant infections (co-infections by histomonads and bacteria (e.g. *Escherichia coli, Clostridium perfringens*) predispose to disease). Histomonad trophozoites feed on solutes and particulate material in the caecal mucosa, while amoeboid forms invade tissues and feed on host tissues. Trophozoites first infect the caecal wall causing small punctate ulcers in which the organisms multiply. The ulcers grow and coalesce resulting in the mucosa becoming inflamed, thickened and necrotic, with dysbiosis causing increased permeability. The caeca become grossly enlarged and haemorrhagic, eventually developing a hard caseous adherent core. Amoeboid stages may then penetrate the mucosa and pass along the portal circulation to the liver and other organs (lung, kidney and spleen in severe cases, sometimes pancreas, heart, brain and bursa). Parasites cause necrotic foci which grow peripherally to produce characteristic (pathognomic) circular depressed lesions up to 1 cm in diameter. The lesions appear as yellow-green necrotic areas with grey peripheral margins and radial streaks (commonly known as 'bulls-eye target' lesions). Lesions are characterized by hyperaemia, haemorrhages, necrosis, serous exudates and infiltrations of macrophages, lymphocytes and multinucleated giant cells.

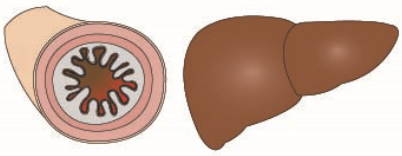
Infected birds exhibit reduced food intake, reduced weight gain, increased thirst, depression, drooping wings, ruffled feathers, yellowish diarrhoea, blackened heads (often due to secondary bacterial infection), and death. Clinical signs may develop within 7-12 days of infection, and most mortalities occur 1-2 weeks later. Surviving birds recover as the necrotic lesions are repaired by lymphocytic cells and fibroblasts, often with scarring. The caseous caecal plug contracts and is expelled allowing the caeca to return to normal size. Recovered birds exhibit a strong protective immunity (involving humoral and cellular responses) but may continue to harbour organisms for some time (premunition). Young turkeys rapidly succumb to infection (mortalities sometimes reaching 80-100%), whereas older birds develop a chronic wasting disease. Chickens and other birds are more resistant to the pathogenic effects of the parasite but may harbour subclinical infections and act as reservoirs of infections for turkeys.

Developmental cycle and mode of transmission: Histomonads do not form environmentally-resistant cysts, with both trophozoites and amoeboid forms being fragile and not surviving long outside of host (only up to 6 hours on feathers and feed, and up to 9 hours in nonchlorinated water and faecal droppings). It was early discovered that caecal worms (*Heterakis gallinarum*) may be involved in transmission cycles as workers were unable to initiate disease in the absence of caecal worms. It was found that histomonads had adopted a unique mode of transmission using caecal nematodes as intermediate hosts. Nematodes feeding on infected host tissues ingest histomonads which then invade their ovaries and infect developing worm eggs. The nematodes then pass infected eggs which are excreted in host faeces. The worm eggs, and their hitch-hiking histomonads, may survive for long periods in the external environment before they are taken up by foraging birds. Once ingested, the worm eggs hatch in the intestines, releasing both worm larvae and histomonads to initiate infections. Chickens are excellent hosts for caecal worms and act as reservoirs of infection, while other birds are poor hosts for caecal worms, but may still act as asymptomatic carriers of infection. It has also been demonstrated that infected worm eggs may be taken up by a small number of invertebrates (including earthworms, houseflies, darkling beetles, and lesser mealworms) which act as paratenic (transport) hosts. Infected worm eggs may survive in these hosts for some time (up to 2 years in earthworms) and remain infective to birds feeding on paratenic hosts. Indeed, infections can accumulate in earthworms and be transmitted to birds, especially following periods of rain when earthworms come to the soil surface and are readily consumed. There is also some evidence that histomonads may be directly transmitted between birds, not by oral infection (as they rarely survive the low pH of the ventriculus unless protected within worm ova), but by the phenomenon of cloacal drinking which transfers materials from the vent area into the caeca through waves of reverse peristalsis (this is a natural process in turkeys whereby the cloaca is stimulated to take up materials from the environment to help build the immune system). Horizontal transmission is thought to be aided by comingling and close contact (huddling behaviour) by turkeys, particularly on moist litters.

Differential diagnosis: Infections are frequently diagnosed on the basis of history, clinical signs and necropsy findings (especially the pathognomonic ‘bulls-eye target’ lesions). Faecal samples and mucosal scrapings can be examined for live parasites using wet mounts, warm stages and high contrast microscopy. Tissue samples taken at necropsy may be processed for histological examination for lesions containing clusters of histomonads following staining with haematoxylin and eosin, periodic acid-Schiffs, Giemsa and Grocott’s silver stains. Histomonads can be cultured *in vitro* from suspect caecal and liver samples using Dwyer’s medium or modified medium 199 with serum supplements. Parasites have also been cultured *in vivo* in turkeys by the intracloacal inoculation of infected liver, caecal tissues or suspensions of cultivated trophozoites. Immunological techniques (enzyme immunoassays) have been developed to detect specific host antibodies and parasite antigens in clinical samples, but their sensitivity and specificity are unknown. More recently, molecular biological techniques have been used to diagnose infections and characterize parasite isolates following the polymerase chain reaction (PCR) amplification of nuclear gene sequences (18S ribosomal RNA, internal transcribed spacers (ITS) 1 and 2, beta-tubulin, alpha-actinin 1, RNA polymerase II subunit RPB1).

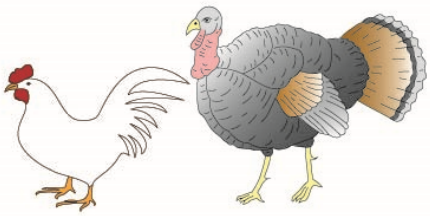
Treatment and control: A range of chemicals have been used to treat histomonad infections in poultry. Early studies using trivalent arsenicals (arsenious acid, atoxyl, neoarsphenamine, tryparsamide) and pentavalent arsenicals (nitarsone, carbasone, roxarsone) gave inconsistent results, while subsequent studies using nitroimidazoles (dimetridazole, metronidazole, ornidazole, tinidazole), nitrofurans (furazolidone), thiametroimidines (enheptin-T) and thiazoles (nithiazide, nitrothiazole) were more effective, although some resistance to metronidazole was observed. However, many drugs are no longer used as feed additives in poultry production due to concerns over toxicities and food residues. There are few other candidates, although an aminoglycoside antibiotic (paromomycin) exhibited reasonable prophylactic activity and some phytochemicals (herbal extracts, dietary supplements) gave good *in vitro* results but not *in vivo.* Fortunately, anthelmintic treatment with benzimidazoles (mebendazole, albendazole, fenbendazole) are very effective against caecal nematode infections, therefore depriving histomonads of their major mode of transmission. Routine treatment of commercial poultry flocks with anthelmintics has drastically reduced the incidence of histomoniasis. Preventive measures employed to further reduce parasite transmission include regular health surveillance (screening birds, quarantining new introductions, culling infected birds, burning or burying bodies), strict hygiene (disinfecting equipment, feeders, waterers, roosts), good sanitation (remove contaminated litter, avoid damp soils), biosecurity (excluding paratenic hosts), and good husbandry (separate cohorts, avoid co-mingling (especially of chickens and turkeys) and reduce stressors (maintain nutrition and avoid over-crowding). Experimental vaccination studies have shown some promise with parasite strains attenuated by serial passage producing some protection when administered orally or cloacally, but no commercial vaccines have yet been developed.

Histomonas

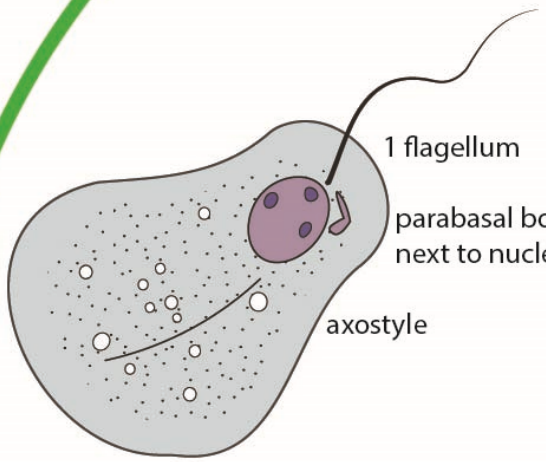


intestines, liver
(ulceration, necrosis,
infectious enterohepatitis
[blackhead disease])

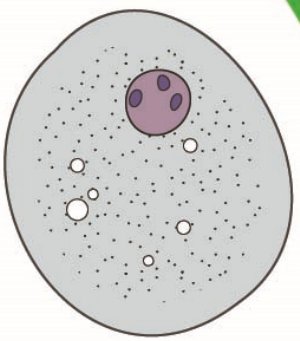
division by
longitudinal
binary fission



Vertebrate Hosts
(birds, esp.
poultry)

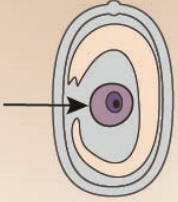


flagellated lumen
forms (5-30 µm)



nonflagellated (amoeboid)
tissue forms (8-19 µm)

trophozoites ingested
by caecal worms invade
ovaries and infect
worm eggs

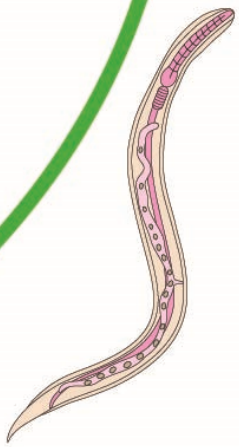


infected worm
eggs ingested

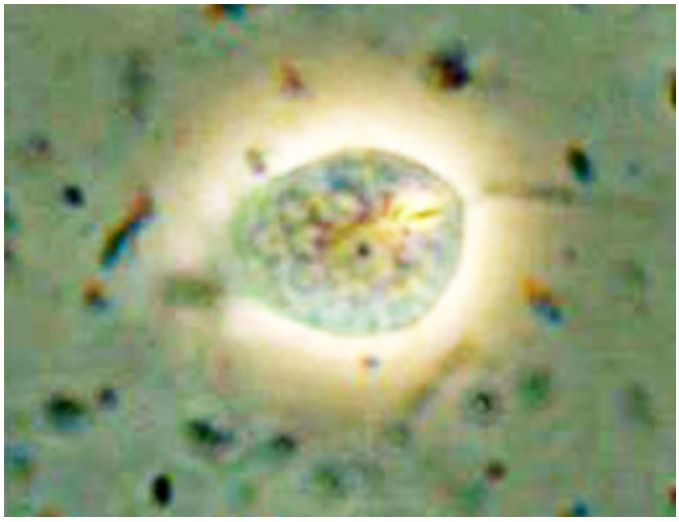
infected worm
eggs excreted

Paratenic Hosts
(earthworms)

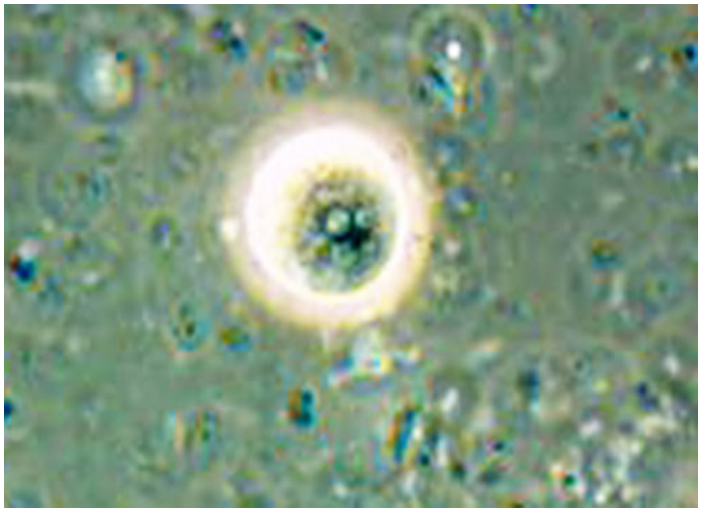
indirect transmission when infected worm eggs
in external environment (or in paratenic hosts)
are ingested by birds



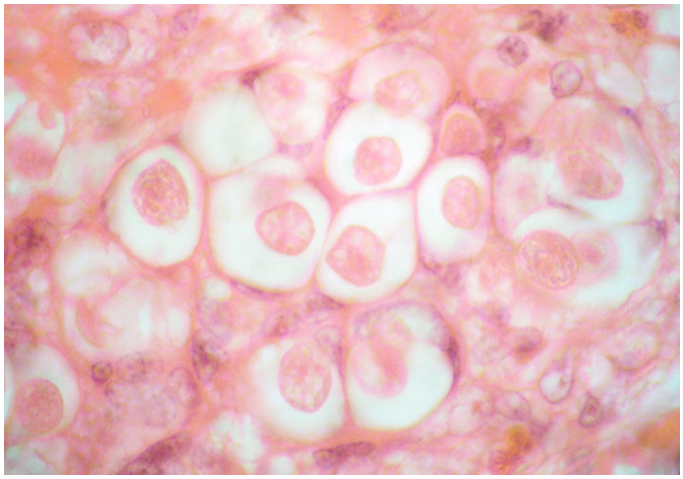
Intermediate Hosts
(caecal worm [*Heterakis*])



Histomonas flagellated trophozoite



Histomonas amoeboid trophozoite



Histomonas liver lesion in chicken