Besnoitia
(protist: apicomplexan)

Overview

Protists are single-celled organisms with membrane-bound nuclei (eukaryotes). One protistan supergroup known as SAR comprises the Stramenopiles (with heterokont flagella), Alveolata (with cortical alveoli) and Rhizaria (with fine pseudopodia). Three major alveolate groups are recognized: ciliates, apicomplexans and dinoflagellates. Apicomplexan cells possess a distinctive apical complex of organelles, comprising a conoid, polar ring, rhoptries, micronemes and subpellicular microtubules, which facilitate entry into host cells as they are obligate intracellular parasites for most of their life-cycles. There are three main apicomplexan groups: gregarines, coccidia and haematozoa. Coccidia form non-motile resistant oocysts that contain infective sporozoites usually confined within secondary spores (sporocysts). Tissue cyst-forming coccidia have heteroxenous (two-host) life-cycles alternating between enteric stages in predators (definitive hosts) and encysted stages in prey (intermediate) hosts. *Besnoitia* spp. form large globular cysts containing numerous bradyzoites (= cystozoites) predominantly in fibroblasts of domestic and wild ruminants and some marsupials. The cysts have exceptionally thick cyst walls and the host cell nucleus is enclosed within the cyst wall. Merogony (= schizogony), gamogony (male microgametes fertilize female macrogametes) and oocyst formation occurs in the intestinal epithelia of cats. Sporogony occurs exogenously and mature oocysts contain 2 sporocysts each with 4 sporozoites (1:2:4 configuration). Different species exhibit relative strict host specificity, with particular predator-prey combinations hosting individual species.

Classification:

Domain:	Eukaryota (membrane-bound nucleus)
Supergroup:	SAR (Stramenopiles + Alveolata + Rhizaria)
Group:	Alveolata (with cortical alveoli)
Phylum:	Apicomplexa (with apical complex, all parasitic, sexual development (gamogony))
Class:	Coccidiomorphea [Conoidasida] (with conoid)
Subclass:	Coccidia [Coccidiasina] (small intracellular gamonts)
Order:	Eucoccidiorida (cyclic merogony (schizogony), gamogony, sporogony)
Suborder:	Eimeriorina (no syzygy, many microgametes)
Family:	Sarcocystidae (heteroxenous, oocysts with two sporocysts, tissue cyst formation in intermediate host)
Subfamily:	Toxoplasmatinae (metrocytes not present, thin cyst walls)
Genus:	*Besnoita* (tissue cyst-forming coccidian parasites of mammals/reptiles)
Species:	various species cause lesions/cysts in animals

Parasite biodiversity and host range: Protists are unicellular eukaryotes that move using undulipodia (flagella or cilia), pseudopodia (false-feet) or a unique gliding motion. Cells with different modes of locomotion do not form separate monophyletic assemblages as previously thought, but rather are distributed across several disparate supergroups (as evidenced by recent molecular phylogenetic analyses). One protistan supergroup known as SAR comprises the Stramenopiles (with heterokont flagella), Alveolata (with cortical alveoli) and Rhizaria (with fine pseudopodia). Three diverse alveolate groups are recognized: Ciliophora (with cilia), Dinoflagellata (with flagella) and Apicomplexa (with gliding motion, some also with flagellated microgametes). Over 4,000 species of Apicomplexa have been described as obligate parasites from vertebrate and invertebrate hosts. At some stage in their development, these possess unique cytoskeletal and membrane-bound organelles (conoid, rhoptries, micronemes, subpellicular microtubules) forming an apical complex that facilitates host cell invasion. Apicomplexans undergo cyclic development involving up to three different divisional processes: asexual merogony (schizogony) either by fission (splitting of maternal cell) or endogeny (internal formation of daughter cells); gamogony involving formation of gametes (macrogametes = female, microgametes = male) which undergo fertilization to recombine by fusion (syngamy) with or without paired alignment (syzygy); and sporogony (formation of infective sporozoites).

Three main apicomplexan groups are recognized: haematozoa, gregarines, and coccidia. Haematozoa are small blood-borne parasites in vertebrates which complete their development in blood-sucking invertebrate vectors; with pleomorphic haemosporidia being transmitted by insects and pear-shaped piroplasms being transmitted by ticks. Gregarines are lumen-dwelling parasites that form large extracellular (sometimes septate) gamonts with an anterior holdfast organelle (mucron or epimerite) used to attach to the gut or body cavity of invertebrates. Coccidia are tissue-invading parasites that form small intracellular gamonts (lacking a mucron or epimerite) and most species undergo sexual reproduction by anisogamous fusion without syzygy forming non-motile resistant spores (oocysts) containing infective sporozoites usually confined within secondary spores (sporocysts). Three groups of coccidia are recognized: coelotrophiid coccidia in marine annelids; adeleid coccidia in marine and terrestrial animals (including blood parasites paradoxically known as 'haemogregarines' in reptiles and amphibians with leech or arthropod vectors); and eimeriid coccidia in vertebrates. Many eimeriid coccidia are monoxenous gut parasites undergoing faecal-oral transmission, but some are heteroxenous alternating between enteric stages in predators and encysted stages in prey (there are also a few enigmatic 'haemococcidia' in the blood of reptiles and birds).

Higher taxonomy	Family	Genera	Hosts	Site	Trans-mission*
Class: Gregarinomorphea (gregarines, trophonts with specialized attachment epimerite or mucron, syzygy)					
Subclass: Cryptogregaria (epicellular parasites of vertebrates with feeder organelle but lacking apicoplast)					
	Cryptosporidiidae (naked sporozoites)	*Cryptosporidium*	vertebrates	gut, lungs	direct (f-o)
Class: Coccidiomorphea [Conoidasida] (with conoid)					
Subclass: Coccidia [Coccidiasina] (small intracellular gamonts)					
Order: Eucoccidiorida (cyclic merogony (schizogony), gamogony, sporogony)					
Suborder: Adeleina (syzygy, 1-4 microgametes)	Haemogregarinidae (ookinete, gamonts in blood cells, invertebrate vectors)	*Haemogregarina*	reptiles, amphibia, fish	tissues, blood	indirect (v-b)
		Hepatozoon	mammals, reptiles	tissues, blood	indirect (v-b)
	Klossiellidae (sporocysts)	*Klossiella*	mammals	kidney	direct (f-o)
Suborder: Eimeriorina (no syzygy, >4 microgametes)	Eimeriidae (monoxenous, endogenous merogony and gamogony, exogenous sporogony)	*Caryospora*	birds, reptiles	gut	direct (f-o)
		Cyclospora	mammals, reptiles	gut	direct (f-o)
		Isospora	birds, reptiles	gut	direct (f-o)
		Eimeria	vertebrates	gut, tissues	direct (f-o)
		Epieimeria	fish	gut	direct (f-o)
		Goussia	fish	gut	direct (f-o)
	Sarcocystidae (heteroxenous, 1:2:4 oocyst:sporocyst:sporozoite configuration)				
	subfamily Cystoisosporinae (monozoic cysts)	*Cystoisospora* (no Stieda bodies)	carnivores, omnivores	gut, tissues	direct (f-o), indirect (p-p)
	subfamily: Sarcocystinae (thick-walls, metrocytes)	*Sarcocystis* (*Frenkelia*)	mammals, birds, reptiles	gut, muscles	indirect (p-p)
	subfamily: Toxoplasmatinae (thin-walled cysts without metrocytes)	*Besnoita*	mammals, reptiles	gut, tissues	indirect (p-p)
		Hammondia	mammals	gut, tissues	indirect (p-p)
		Neospora	herbivores, dogs	gut, tissues	indirect (p-p)
		Toxoplasma	vertebrates, cats	gut, tissues	indirect (p-p)
Class: Aconoidasida (asexual stages without conoid)					
Subclass: Haematozoa (clade of vector-borne spore-forming haemo-protozoa)					
Order: Haemosporida (pleomorphic blood stages, insect vectors, motile ookinete)	Plasmodiidae (schizogony in tissues then blood cells, haemozoin pigment)	*Plasmodium*	mammals, birds, reptiles	liver, erythrocytes	indirect (v-b)
	Haemoproteidae (schizogony in tissues, haemozoin pigment)	*Haemoproteus*	birds	endothelia, erythrocytes	indirect (v-b)
	Leucocytozoidae (schizogony in tissues, no haemozoin pigment)	*Leucocytozoon* (*Akiba*)	birds	tissues, leucocytes	indirect (v-b)
Order: Piroplasmorida (pear-shaped blood stages, tick vectors)	Babesiidae (merogony in erythrocytes, trans-stadial + trans-ovarian transmission)	*Babesia*	mammals	erythrocytes	indirect (v-b)
	Theileriidae (merogony in leucocytes, trans-stadial transmission in ticks)	*Theileria*	ruminants	leucocytes, erythrocytes	indirect (v-b)

* f-o = faecal-oral transmission; p-p = predator-prey transmission; v-b = vector-borne transmission.

Numerous species of coccidia have been described from a wide range of vertebrate and invertebrate hosts. Some 50 genera have been classified in 11 families in the suborder Eimeriorina, including nine genera in the family Sarcocystidae. These apicomplexans are often referred to as cyst-forming coccidia, as they undergo gamogony and sporogony producing oocysts (1:2:4 oocyst:sporocyst:sporozoite configuration) in the intestines of carnivorous definitive hosts (DHs) as well as merogony and tissue cyst formation in the tissues of omnivorous or herbivorous intermediate hosts (IHs). They have heteroxenous (two-host) life-cycles with cyclic transmission between predatory animals and their prey. Two main subfamilies are recognized mainly on the basis of differences in cyst development (metrocytes present or absent) and site of oocyst sporulation (endogenous or exogenous). Members of the subfamily Sarcocystinae form cysts with metrocytes within the tissues of their intermediate hosts, and their oocysts sporulate endogenously before being voided from the definitive host. Members of the subfamily Toxoplasmatinae form cysts without metrocytes within the tissues of their intermediate hosts, and their oocysts sporulate exogenously after being voided from the definitive host. A third subfamily has recently been added with the discovery that some *Isospora* spp. in mammals form encysted monozoic stages (cystozoites) in the tissues of paratenic (transport) hosts (esp. rodents), prompting their classification with the tissue cyst-forming coccidia under the name *Cystoisospora* in the new subfamily Cystoisosporinae.

Parasite genera	**No. spp.**	**Life-cycle**	**Definitive Hosts (DH) Intermediate Hosts (IH) Paratenic Hosts (PH)**	**Oocyst configuration***
Family: Sarcocystidae (3 subfamilies)				
Subfamily: Cystoisosporinae (monozoic cysts in PH, sporocysts without Stieda bodies)				
Cystoisospora	50	heteroxenous	vertebrate DH (carnivores, primates), vertebrate PH (mammals, birds)	1:2:4
Subfamily: Sarcocystinae (metrocytes, endogenous sporulation)				
Sarcocystis (incl. *Frenkelia*)	135	heteroxenous	vertebrate DH (predatory mammals, birds, reptiles), vertebrate IH (mammals, birds, reptiles)	1:2:4
Subfamily: Toxoplasmatinae (no metrocytes, exogenous sporulation)				
Toxoplasma	1	heteroxenous	vertebrate DH (felids), vertebrate IH (mammals), invertebrate PH (annelids, insects)	1:2:4
Hammondia	3	heteroxenous	vertebrate DH (canids, felids), vertebrate IH (mammals)	1:2:4
Neospora	2	heteroxenous	vertebrate DH (canids), vertebrate IH (mammals)	1:2:4
Besnoitia	7	heteroxenous	vertebrate DH (felids), vertebrate IH (mammals, reptiles), possibly invertebrate PH (insects)	1:2:4
Hyaloklossia	1	monoxenous	vertebrates (amphibians)	1:2:4
Nephroisospora	1	monoxenous	vertebrates (bats)	1:2:4

*1:2:4 = one oocyst contains 2 sporocysts, each sporocyst contains 4 sporozoites

The genera *Toxoplasma*, *Hammondia*, *Neospora* and *Besnoitia* are obligatory or facultatively heteroxenous with cyclic predator-prey transmission usually between carnivorous definitive hosts (DH) and herbivorous intermediate hosts (IH). Transmission from IH to DH occurs carnivorism (predator consuming cysts in tissues of prey) and transmission from DH to IH occurs via faecal-oral contamination (excretion of oocysts/sporocysts in faeces of predators to contaminate foodstuffs of prey). Several species are also di-heteroxenous (less common term di-homoxenous), meaning that infections can be passed horizontally between intermediate hosts by carnivorism (ingestion of tissue cysts in IH or PH) or vertically from mother to offspring (via transplacental or transmammary infection). These heteroxenous genera within the subfamily Toxoplasmatinae form tissue cysts without metrocytes, the bradyzoites undergo asexual division before gamete formation and the oocysts sporulate exogenously after being voided. More recently, encysted stages of two monoxenous genera (*Hyaloklossia* and *Nephroisospora*) have been found in the tissues of amphibians and bats, prompting their placement in the subfamily Toxoplasmatinae.

The genus *Besnoitia* comprises some 13 species which form large cysts (sometimes grossly visible) in the connective tissues (primarily fibroblasts) of a range of intermediate hosts (ruminants, horses, opossums, rodents and lizards). The intracellular cysts (sometimes called pseudocysts) lack subdivisions and are surrounded by thick laminated nucleated cyst walls. Several species have been associated with high morbidity and low mortality in ruminants and horses, with disease endemic in some tropical and subtropical areas, and sporadic in other areas. Experimental studies have revealed the definitive hosts for 5 *Besnoitia* spp. to be cats, which excrete small unsporulated isosporid oocysts in their faeces (oocyst:sporocyst:sporozoite configuration of 1:2:4 resembling those of *Toxoplasma*). Transmission between hosts has been demonstrated following oocyst ingestion (faecal-oral transmission from DH to IH), tissue cyst ingestion (predator-prey transmission from IH to DH) as well as mechanically by blood-sucking arthropods (vector-borne transmission between IHs).

***Besnoitia* species**	**Intermediate hosts (IH)**		**Definitive hosts (DH)**	**Distribution**
	Species	**Location**		
B. akodoni	Rodentia: cricetid (montane grass mouse), experimental infection in murid (house mouse, gerbil)	abdominal muscles	unknown	Brazil
B. bennetti	Perissodactyla: equid (horse, mule, burro, donkey)	eyes, mouth, testes, cutis, subcutis	unknown	Africa, Europe, North America

B. besnoiti (type species)	Artiodactyla: bovid (cattle, zebu, water buffalo, sheep, goat, wild goat, blue wildebeest, impala, kudu, duiker), cervid (deer); Lagomorpha: leporid (rabbit); Rodentia: caviid (guinea pig), murid (house mouse, jird), sciurid (yellow ground squirrel, marmot), cricetid (golden hamster)	cutis, subcutis connective tissue, fascia, serosae, mucosae	Carnivora: felid (cat, African wild cat)	South America, Southern Europe, Africa, Asia
B. caprae	Artiodactyla: bovid (goat, sometimes sheep)		unknown	Africa, southern Europe, New Zealand
B. darlingi (syn. *B. panamensis, Sarcocystis darlingi*)	Didelphimorphia: didelphid (black-eared opossum, Virginia opossum, big-eared opossum, Derby's woolly opossum, grey four-eyed opossum); Primates: callitrichid (Geoffroy's tamarin); Chiroptera: phyllostomid (Seba's short-tailed bat); Rodentia: sciurid (red-tailed squirrel, variegated squirrel), murid (house mouse), cricetid (golden hamster); Sauria: corytophanid (common basilisk, brown basilisk), teiid (giant ameiva, tiger ameiva, delicate ameiva)	striated muscles, connective tissue	Carnivora: felid (cat)	Americas
B. jellisoni	Rodentia: murid (house mouse, brown rat), cricetid (North American deer mouse, cloud forest grass mouse, golden hamster, voles), heteromyid (kangaroo-rats), sciurid (ground squirrels), caviid (guinea pig); Didelphimorphia: didelphid (Virginia opossum)	connective tissue, serosae	unknown	North America
B. neotomofelis	Rodentia: cricetid (Southern Plains woodrat), plus experimental infection in murid (house mouse, brown rat); di-heteroxenous (IH-IH) transmission via carnivorism	muscles, subcutis	Carnivora: felid (cat)	North America
B. oryctofelis	Lagomorpha: leporid (rabbit), plus experimental infection in Rodentia: murid (mice, gerbil); Lagomorpha: leporid (rabbit); Carnivora: felid (cat)	tissues	Carnivora: felid (cat)	South America
B. panamensis	Sauria: corytophanid (brown basilisk), teiid (giant ameiva), plus experimental infection in Rodentia: murid (mouse)		unknown	Panama
B. paraguayensis	Cingulata: dasypodid (nine-banded armadillo)		unknown	South America
B. sauriana	Sauria: corytophanid (brown basilisk)		unknown	Belize
B. tarandi	Artiodactyla: cervid (reindeer, caribou, mule deer), bovid (musk ox)	fibrous connective tissue, skin (head, legs, scrotum) [alopecia, thickening, ulceration, 'cornmeal' disease]	unknown	Finland, Canada
B. wallacei (syn. *Isospora wallacei*)	Rodentia: murid (house mouse, brown rat, black rat, Polynesian rat, Mongolian gerbil), cricetid (Japanese grass vole); Lagomorpha: leporid (rabbit)	connective tissue	Carnivora: felid (cat)	Australasia, Hawaii

Parasite morphology: *Besnoitia* spp. form 5 different types of developmental stages: meronts and tissue cysts in intermediate hosts (IH); and meronts, gamonts and oocysts in definitive hosts (DH). Meronts (often called schizonts) in IH are small asexual multiplicative forms found in host cells (fibroblasts, macrophages, endothelial cells) as small basophilic rounded bodies (10-25 μm) that undergo internal division (endogeny) to produce numerous (4-20) daughter merozoites (often called tachyzoites) that are characteristically crescent- or banana-shaped (5-8 x 1-2 μm). Tissue cysts are larger encapsulated round-elongate stages formed within host cells (often fibroblasts). They may range in size from microscopic (~200 μm diameter) to macroscopic (up to several mm in diameter). Cysts have thick collagenous walls (10-100 μm thick) that also contain numerous hypertrophic host cell nuclei, and *Besnoitia* cysts have thick secondary cyst walls (unlike most other cyst-forming sporozoa). The cysts undergo repeated internal division to contain thousands of bradyzoites (cyst merozoites, cystozoites) which are elongate crescent-shaped cells (9 x 1.5 μm). Ultrastructural studies reveal bradyzoites to possess a conoid, rhoptries, micronemes and to be bound by pellicular membranes with 22 subpellicular microtubules. Meronts in DH are asexual stages found in subepithelial locations in the intestines, sometimes in extraintestinal tissues. They appear as round basophilic bodies (10-45 μm) that divide asexually to produce numerous (4-30) merozoites. Sexual development (gamogony) occurs in goblet cells in the small intestinal epithelium, with sexually-dimorphic gamonts producing gametes. Macrogamonts (female) develop into rounded macrogametocytes (12-18 μm) which mature to form single macrogametes that are uninucleate egg-like cells with many cytoplasmic vacuoles. Microgamonts (male) form basophilic microgametocytes (10-15 μm) that divide internally to produce multiple (4-8) microgametes that are small slender biflagellated sperm-like stages (4 x 1 μm). Fertilization occurs by fusion of macro- and micro-gametes producing zygotes that form oocysts which are excreted unsporulated. Developing oocysts are surrounded by thin membranous walls and they undergo internal sporulation (sporogony) to produce 2 sporocysts, each of which produces 4 sporozoites (resulting in *Isospora*-like oocysts with 1:2:4 oocyst:sporocyst:sporozoite configuration). The oocysts are ovoid (11-17 x 11-12 μm) and lack micropyles and polar granules. The sporocysts are elliptical (8-11 x 6-8 μm) and lack Stieda bodies. The sporozoites are slender crescent-shaped cells (4-6 x 2 μm) which often have refractile bodies.

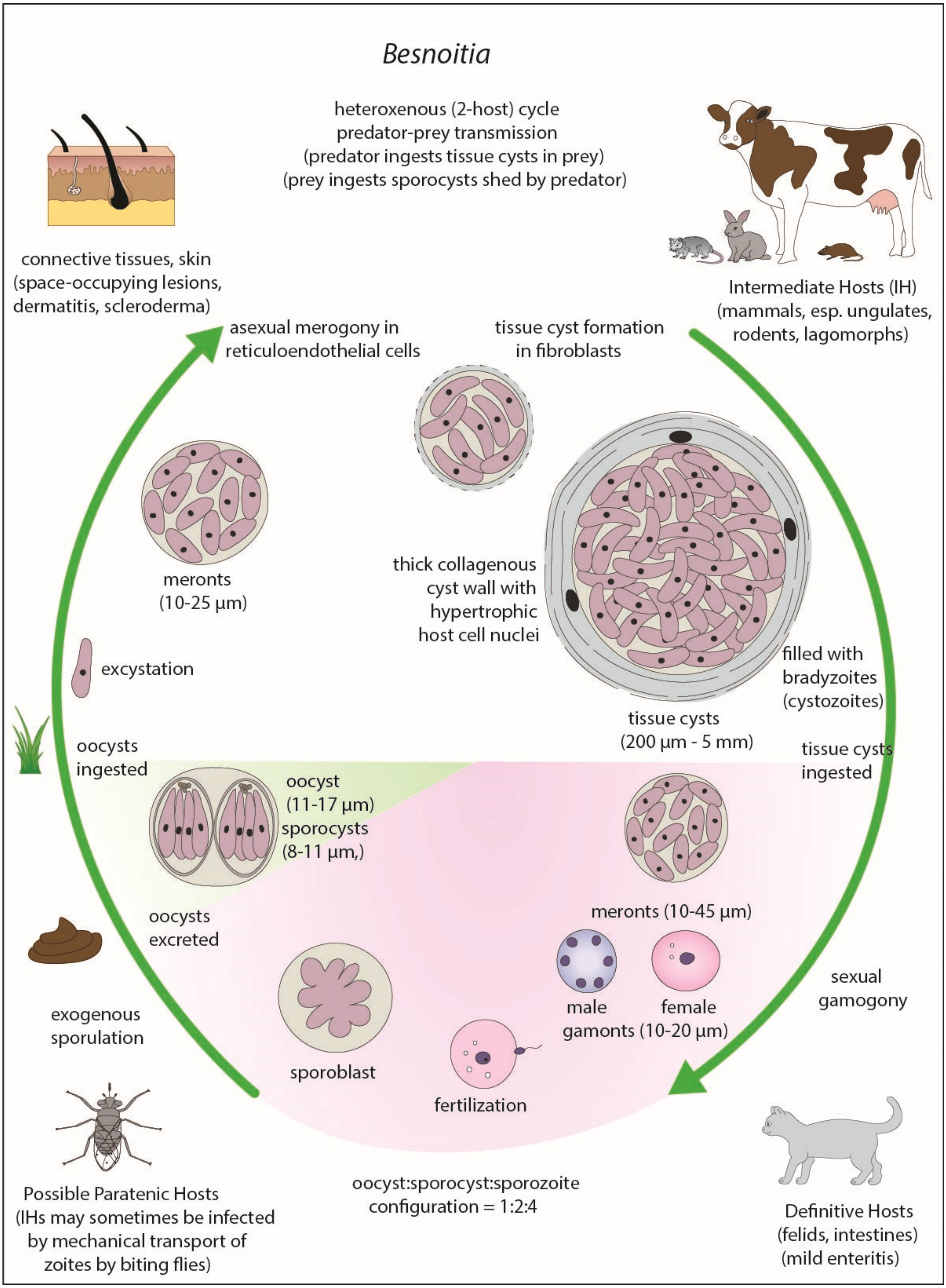
Site of infection: Asexual development of the parasite takes place in intermediate hosts (usually herbivores), with meronts forming in vascular endothelial cells or connective tissues cells (fibroblasts, macrophages) in various host tissues and organs, followed by cyst formation in connective tissues (mainly fibroblasts), particularly in the skin, mucous and serous membranes (e.g. conjunctiva, mesentery) and scrotum. Infections by tissue cysts have been recorded in small but diverse array of vertebrates, including 15 artiodactylan species (mainly bovids, some cervids), 4 perissodactylans (equids), 20 rodents (caviids, cricetids, heteromyids, murids, sciurids), 5 opossums (didelphids), one lagomorph (leporid), one bat (phyllostomid), one cingulate (dasypodid), one primate (callitrichid) and 5 lizard species (corytophanids, teiids). Both asexual and sexual reproduction of the parasite occurs in definitive hosts (some carnivores), with one merogonous generation developing in the lamina propria of the small intestines; followed by gamete formation, fertilization and oocyst formation in epithelial cells (usually goblet cells). In those *Besnoitia* spp. for which life-cycles have been determined (namely, *B. besnoiti, darlingi, neotomofelis, oryctofelis, wallacei*), sexual development apparently occurs exclusively in felids (cats).

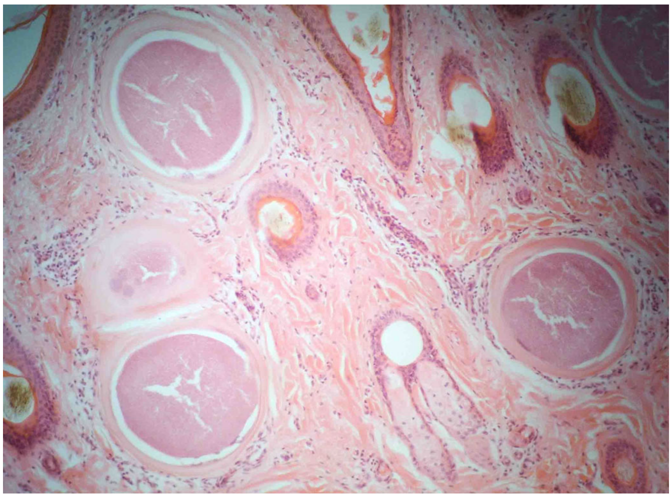
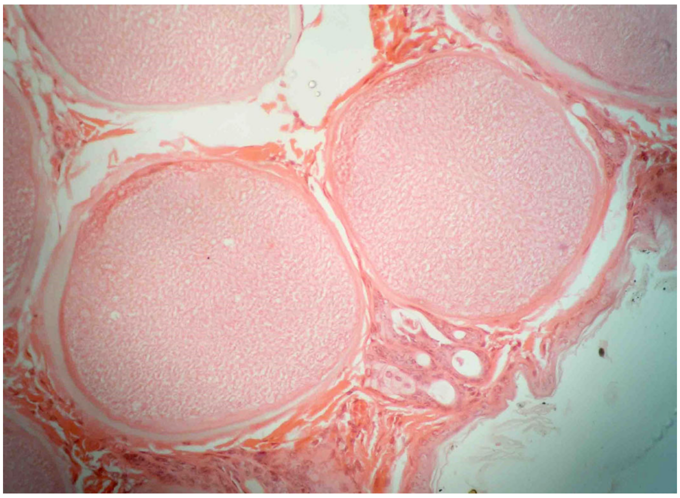
Pathogenesis: Many infections are asymptomatic and often go unnoticed, but some may cause acute or chronic clinical disease known as besnoitiosis (originally called globidiosis) which varies in severity from mild to fatal. In cattle, disease (also known as bovine elephantiasis, bovine anasarque, or elephant skin disease) occurs in 2 stages: an acute 'anasarca' stage associated with painful swellings; and a chronic 'scleroderma' stage associated with thickening and hardening of the skin. Acute disease is caused by meronts proliferating in vascular endothelial cells causing focal lesions, necrosis of venules and arterioles, thrombosis and vasculitis. Small white elevated macules may be observed on the scleral conjunctiva and nasal, pharyngeal and laryngeal mucosa. Animals may develop transient hyperthermia or fever (40-41.6°C) for 3-10 days with warm painful swellings (anasarca) occurring mainly in ventral parts of the body, but oedema contributing to respiratory disorders, orchitis and limps. Other signs include congestive mucosa, lacrimation, rhinitis with increased nasal discharge (initially serous, but may become mucopurulent and contain blood), tachycardia, swollen lymph nodes, diarrhoea, anorexia, weight loss, depression and photophobia. Acute signs may last for 1-3 weeks and then subside as the parasites begin cyst formation exhibiting a special tropism for connective tissues throughout the body. Chronic infection then develops as cysts grow, fill with bradyzoites and become encapsulated by thick collagenous cyst walls. Cysts occur in the dermis, subcutis, fascia, mucosa of the upper respiratory tract, pharynx, conjunctiva, lower reproductive tract in females and testes and epididymis in males and become fully developed within 4-7 weeks of infection. Their presence may cause debilitating disease characterized by cellular destruction, inflammation (including granulomatous reactions against ruptured cysts) and gross disfigurement of the skin with alopecia, dermatitis, eczema, hyperpigmentation, hyperkeratosis and scleroderma (hardened thickened skin). Heavy infections may result in wrinkled corrugated skin thrown into folds around the neck, shoulders, rump and carpal/tarsal areas, with nodules, scars and cracks allowing secondary bacterial infections and myiasis. Other signs include focal disseminated myositis, periostitis, endostitis, lymphadenitis, arteritis, perineuritis, pneumonia, periorchitis, orchitis, epididymitis, anorexia, emaciation, lethargy, and death (reports of up to 10% mortalities). Males may also become sterile due to aspermatogenesis caused by necrotizing orchitis with testicular atrophy and induration. Chronic infections have been associated with production losses in livestock, through unsightly lesions, disfigurement, damaged hides, reduced milk production, infertility/sterility in males, and sometimes abortion due to persistent fever in dams (but parasites do not cross the placenta). Infections in goats mostly involve chronic disease with dermatitis of the ventral abdomen and legs varying from mild thickening with superficial scaling to marked scleroderma with hyperpigmentation and sometimes serous discharges. Infections in equids may cause small white nodules on the sclera, nares, soft palate, pharynx, larynx causing nasal discharges and inspiratory dynspoea as well as skin lesions on the ventral abdomen and legs often associated with exercise intolerance. Unusual cases have been reported in kangaroos suffering, and occasionally dying, from nose-bleeds (epistaxis), with parasites detected in nasal flushes.

Developmental cycle and mode of transmission: Studies have shown that some *Besnoitia* spp. have heteroxenous life cycles where cysts in herbivorous intermediate hosts are infective to carnivorous definitive hosts resulting in oocyst production. Cats have been found to act as definitive hosts for several species (*B. besnoiti, darlingi, neotomofelis, oryctofelis, wallacei*) but the life-cycles for the remaining species have yet to be determined. When cats consume infected flesh, the tissue cysts are digested in the gut facilitating the release of the contained bradyzoites which invade the lamina propria in the small intestines. The parasites undergo one asexual generation forming meronts over 4-13 days which produce and release numerous tachyzoites. These cells invade goblet cells in the mucosal epithelium and undergo sexual reproduction ultimately producing macrogametes (female) and microgametes (male) which fuse to form zygotes that differentiate into oocysts. Unsporulated oocysts are excreted with host faeces and undergo exogenous sporulation in 2-3 days, each oocyst forming 2 sporocysts, and each sporocyst forming 4 sporozoites (1:2:4 configuration). The prepatent period (time interval from infection to oocyst production) ranges from 9-14 days, and the patent period (duration of oocyst excretion) ranges from 3-13 days. The oocysts are highly resistant to external environmental conditions and contaminate food and water supplies. When susceptible intermediate hosts ingest infective oocysts, they excyst in the small intestines releasing the contained sporozoites. The sporozoites invade vascular endothelial cells and connective tissue cells (fibroblasts and macrophages) where they undergo asexual proliferation forming meronts that produce numerous tachyzoites. The tachyzoites the invade connective tissue cells (mainly fibroblasts) where they initiate cyst formation around 30-36 days after infection. The cysts mature over several weeks to months, some growing to macroscopic size and containing thousands of bradyzoites. The parasite life-cycle is completed when cysts are consumed by carnivorous definitive hosts. In addition to heteroxenous predator-prey transmission, experimental studies have shown that some *Besnoitia* spp. can be transmitted horizontally between hosts by needle inoculation of tachyzoites or bradyzoites. Because many outbreaks of disease in cattle or goats occur in fly seasons, it has been postulated that biting insects may be important vectors or paratenic hosts. Mechanical transmission by biting arthropods has been mooted for *B. tarandi* and *B. caprae* and demonstrated for *B. besnoiti* via tsetse flies (*Glossina*), stable flies (*Stomoxys*) and horse flies (*Tabanus, Atylotus*). There is no evidence to support transplacental or venereal transmission, despite occasional abortions in pregnant animals (thought to be due to persistent fever) and the high prevalence of scrotal infections in males.

Differential diagnosis: Infections may be diagnosed in intermediate hosts by the direct detection or cultivation of parasites in clinical samples, by the immunological detection of specific host antibodies against parasites, or by the molecular detection of parasite DNA in host materials. Animals may be subject to antemortem clinical examination looking for tissue cysts in subcutaneous tissues, scleral conjunctiva or nasal mucosa. Biopsy material may be collected, including scrapings (skin, scleral conjunctiva, vestibulum vaginae), punch or incisional biopsies, needle aspirates, and blood), and smears or trichinoscopic squash preparations examined for characteristic crescent-shaped bradyzoites. More frequently, tissue cysts are detected at autopsy by gross or microscopic examination. Tissues may be fixed and histological sections examined for parasites following staining with haematoxylin and eosin or Giemsa. Polyclonal and monoclonal antibodies have also been produced against tachyzoites and bradyzoites and used for the immuno-localization of parasites in tissue preparations by fluorochrome or chromogenic labelling. Parasites may be readily cultured *in vitro* one various tissue culture cell lines, including primary bovine umbilical vein endothelial cells (BUVEC), African green monkey kidney cells (CV-1, MARC-145) and bovine macrophages (M617), as well as *in vivo* by needle passage in laboratory mice (including interferon-gamma gene knockout mice) or Mongolian gerbils. Experimental studies have examined changes in haematological and biochemical parameters during infection, noting reduced leukocyte and erythrocyte concentrations, haematocrit, serum albumin, urea, magnesium and calcium concentrations, elevated serum total protein, globulin, total bilirubin, creatinine, creatine kinase, and aspartate transaminase, but most findings could be attributed to inflammation and muscle necrosis. Several immunological techniques (indirect fluorescent-antibody tests, microagglutination tests, enzyme immunoassays) have been developed to detect host antibodies against parasite antigens, with considerable variability observed in their sensitivity and specificity depending on antigen types (tachyzoites/bradyzoites, cultured/harvested cells, whole/subcellular fractions), reagent fidelity (antiserum specificity, immunoglobulin classes, contaminants (e.g. foetal bovine or horse serum used in culture medium) and possible concomitant infections by related cyst-forming sporozoa (notably *Neospora caninum* and *Toxoplasma gondii*). Immunoblot techniques have also been used to detect parasite antigens in host sera, with some variability observed under reducing or non-reducing run conditions. More recently, molecular biological techniques have been applied to the detection and characterization of parasites in blood, biopsy and necropsy samples following the polymerase chain reaction (PCR) amplification of nuclear gene sequences (18S and 5.8S ribosomal DNA, internal transcribed spacer region 1, protein disulphide isomerase) or by genotyping at 6 microsatellite loci (Bt-5,6,7,9,20,21).

Treatment and control: A wide range of chemotherapeutic agents have been used in attempts to treat or prevent *Besnoitia* infections in animals, but few have been effective. Treatments with semimetallic compounds (antimony) and sulphonamide antibiotics (sulfanilamide) were found to suppress cyst development in rabbits, and treatments with tetracycline antibiotics (oxytetracycline) were moderately effective if given early enough in the course of disease. However, the use of more regular antiprotozoal drugs, including thiazolides (nitazoxanide, tizoxanide), sulphonamides (sulfadiazine), bumped kinase inhibitors, triazinones (diclazuril, toltrazuril, ponazuril), quinolones (decoquinate) and naphthoquinones (buparvaquone), were ineffective. Experimental *in vitro* studies found that polyphenolic compounds (curcumin) were effective against *Besnoitia* tachyzoites in tissue culture, but in vivo studies are outstanding. Some enteric infections in cats responded well to treatment with lincosamide antibiotics (clindamycin), but the effect of conventional anti-coccidial drugs on oocyst production remains to be determined. Preventive measures focus on reducing the risks of transmission through health surveillance, biosecurity and herd management. Animals should be regularly tested with screening carried out in intensive systems prior to moving livestock indoors. Infected animals should be isolated and/or culled from herds, and all new replacement stock screened for infections, in quarantine if necessary. It is advisable to maintain closed herds and to avoid co-mingling and sharing pastures. This includes not sharing bulls, but rather to using artificial insemination programs involving seronegative bulls. Farms should endeavour to keep cats away from supplementary food storage areas and to adopt alternative rodent control measures if required. Despite supporting evidence, cattle isolated and protected from arthropod ectoparasites would be less at risk from any horizontal mechanical transmission involving biting insects. The use of indoor traps, repellents (diethyl-meta-toluamide (DEET), pyrethroids), insecticides (novaluron) and insect growth regulators (cyromazine) in pens and feeding sites have helped reduce muscid and tabanid fly numbers and their activity during peak fly season. Several experimental vaccines have been developed using live attenuated cultures of parasites, and they are used in several countries to prevent infections in cattle, especially in imported bulls.


Besnoitia
heteroxenous (2-host) cycle
predator-prey transmission
(predator ingests tissue cysts in prey)
(prey ingests sporocysts shed by predator)
connective tissues, skin
(space-occupying lesions,
dermatitis, scleroderma)
Intermediate Hosts (IH)
(mammals, esp. ungulates,
rodents, lagomorphs)
asexual merogony in
reticuloendothelial cells
tissue cyst formation
in fibroblasts
meronts
(10-25 μm)
thick collagenous
cyst wall with
hypertrophic
host cell nuclei
filled with
bradyzoites
(cystozoites)
excystation
tissue cysts
(200 μm - 5 mm)
oocysts
ingested
tissue cysts
ingested
oocyst
(11-17 μm)
sporocysts
(8-11 μm,)
meronts (10-45 μm)
oocysts
excreted
male
female
gamonts (10-20 μm)
sexual
gamogony
exogenous
sporulation
sporoblast
fertilization
Possible Paratenic Hosts
(IHs may sometimes be infected
by mechanical transport of
zoites by biting flies)
oocyst:sporocyst:sporozoite
configuration = 1:2:4
Definitive Hosts
(felids, intestines)
(mild enteritis)

 *Besnoitia* cysts in cow skin	 *Besnoitia* cysts in cow skin
 *Besnoitia* oocyst from cat faeces	