Klossiella
(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). Klossiellids are adeleorine coccidia which have monoxenous (1-host) life cycles and are commonly found in equids, rodents and marsupials. Parasites undergo merogony (= schizogony), gamogony and sporogony in the kidneys of vertebrate hosts culminating in the formation of numerous sporocysts which are passed with host urine. Most infections appear to asymptomatic, although several species have been associated with renal lesions in horses and dasyurids.

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:	Adeleina (syzygy, 1-4 microgametes)
Family:	Klossiellidae (zygote inactive, sporocysts formed (rather than oocysts))
Genus:	*Klossiella* (parasitic in kidneys of mammals)
Species:	various species cause renal infections in rodents and horses

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, Apicomplexa 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.

Klossiellid parasites are adeleid coccidia with monoxenous (1-host) life cycles. They do not infect erythrocytes but undergo the typical adeleorine developmental cycle of merogony, gamogony and sporogony in the kidneys of vertebrate hosts. Unlike the eimeriid coccidia, klossiellid gametocytes occur in pairs (syzygy) and few microgametes are produced. The resultant zygote is nonmotile and sporulates producing numerous sporocysts which are passed with host urine. Infections have been recorded in various hosts in several tropical and subtropical countries (mainly in mice, rats, guinea pigs, horses, donkeys, burro, zebra, bats). A single species, *K. equi,* occurs in horses and infections have been detected in many countries. In Australia, infections have been detected in native water rats, bandicoots, gliders, wallabies, hare-wallabies, pademelons, bettongs and kangaroos.

Klossiella species	Hosts	Location	Clinical signs	Distribution
K. bettongiae	Diprotodontia: potoroid (eastern bettong)	kidneys		Australia
K. beveridgei	Diprotodontia: macropodid (spectacled hare-wallaby)	kidneys		Australia
K. boae	Serpentes: boid (boa constrictor)	kidneys	enteritis, anorexia	America
K. callitris	Diprotodontia: macropodid (western grey kangaroo)	kidneys		Australia
K. cobayae	Rodentia: caviid (guinea pig)	kidneys		Africa, Europe, North America
K. convolutor	Diprotodontia: pseudocheirid (ringtail possum)	kidneys		Australia
K. dulcis	Diprotodontia: petaurid (sugar glider)	kidneys		Australia
K. equi	Perissodactyla: equid (horse, donkey, burro, zebra)	kidneys		cosmopolitan
K. hydromys	Rodentia: murid (water rat)	kidneys		Australia
K. killicki	Chiroptera: hipposiderid (roundleaf bat), rhinolophid (horseshoe bat)	kidneys		Africa, North America
K. mabokensis	Rodentia: murid (soft-furred mouse, house mouse)	kidneys		Africa
K. muris	Rodentia: murid (house mouse), caviid (guinea pig)	kidneys	tubule necrosis, death	worldwide
K. quimrensis	Peramelemorphia: peramelid (southern brown bandicoot, eastern barred bandicoot, western barred bandicoot)	kidneys	renal coccidiosis	Australia
K. rufi	Diprotodontia: macropodid (red kangaroo)	kidneys		Australia
K. rufogrisei	Diprotodontia: macropodid (Bennett's wallaby)	kidneys		Australia
K. schoinobatis	Diprotodontia: pseudocheirid (greater glider)	kidneys		Australia
K. serendipensis	Diprotodontia: macropodid (swamp wallaby)	kidneys		Australia
K. tejerai	Didelphimorphia: didelphid (opossum, big-eared opossum, Linnaeus's mouse opossum)	kidneys	'ballooning necrosis' of tubular epithelia	South America
K. thylogale	Diprotodontia: macropodid (Tasmanian pademelon)	kidneys		Australia

Parasite morphology: *Klossiella* spp. form 3 different types of morphological stages during their developmental cycles: namely, meronts (or schizonts), gamonts (male and female), and sporonts (producing sporocysts and sporozoites). Meronts appear as small basophilic bodies (8-20 µm) in kidney cells where they undergo internal (endogenous) division to form numerous slender merozoites (3-7 x 0.5-2.0 µm). Most species form 2 meront generations in the kidneys, although one (*K. muris*) only forms one generation, and another (*K. mabokensis*) apparently forms meronts in intestinal villi before invading the kidneys. The first meront generation usually produces 8-12 merozoites, while the second generation meronts usually form 40-60 merozoites, sometimes up to 100. Gamonts appear as sexually dimorphic stages that develop (often in pairs) into macrogametocytes (female) and microgametocytes (male). Macrogametocytes are rounded stages (4-14 µm in diameter) that produce single macrogametes with a central nucleus and a pale-staining vacuolated cytoplasm. Microgametocytes are also rounded stages (8-12 µm in diameter) that produce variable numbers (1-13) of small (6 x 2 µm) slender peripherally-located microgametes that are non-flagellated (*K. muris* produces 1-2 microgametes, *K. cobayae* 2-4, and *K. equi* 8-10). Gamete fertilization produces a non-motile zygote that undergoes a unique sporulation process. Typical oocysts are not formed, but membrane-bound sporonts produce sporoblasts that form sporocysts, which in turn form sporozoites. Early sporonts appear as ovoid (10-18 µm) cytoplasmic masses containing 8 or more peripheral nuclei (each surrounded by a clear halo) and 10-20 small plastin granules. Developing sporonts are irregular in size and shape (40-100 x 30-60 µm) as the enclosed sporoblasts produce numerous (8-40) subspherical-ellipsoidal sporocysts (ranging in size from 18-24 x 12-22 µm). *K. killicki* forms 8 sporocysts, *K. hydromyos* and *K. mabokensis* form 12, *K. tejerai* forms 18, *K. cobayae* forms 20-24, and *K. equi* forms 30-40. Each sporocyst contains numerous (12-35) falciform- to banana-shaped sporozoites (7 x 3 µm) lying side-by-side with a small residual body.

Site of infection: All developmental stages occur in the kidneys of vertebrate hosts, mostly in marsupials (diprotodonts, peramelemorphids, didelphimorphids) but also including equids, bats, rodents, and snakes. Meronts are typically found in glomeruli (Bowman's capsules) and convoluted tubules, but have occasionally been detected in other organs (intestines, liver, lung, spleen, thyroid, lymph nodes, brain, pituitary, thyroid or adrenal glands). Gamonts, sporonts and sporocysts are found within proximal convoluted tubules; and mature sporocysts are shed in the urine.

Pathogenesis: Most *Klossiella* infections are asymptomatic or subclinical and do not cause overt clinical signs, even though parasite development occurs intracellularly within renal cells resulting in their eventual lysis to release parasite progeny. A few species, however, have been associated with mild-severe clinical disease (klossiellosis) characterized by impaired renal function with tubular nephrosis and interstitial nephritis, including *K. muris* in rodents, *K. tekerai* in opossums, and *K. quimrensis* in bandicoots. Heavily-parasitized kidneys developed grossly visible lesions, evident as small grey foci scattered over the cortical surface, often in the region of the cortico-medullary junction. The grey foci were found microscopically to be areas of cellular necrosis and proliferation. Infections did not cause inflammatory responses in the immediate vicinity of the parasites, but lymphocytes, histiocytes and sometimes fibroblasts did infiltrate the interstitium, notably surrounding blood vessels. Developing schizonts caused significant enlargement of host endothelial cells with compression of surrounding structures, and developing sporonts sometimes occluded the lumina of convoluted tubules. Infections by *K. equi* in horses have occasionally been associated with multifocal tubular necrosis and, in the case of tubular rupture, with interstitial infiltrates of lymphocytes and plasma cells, but renal function essentially remained normal (although a few sporadic cases of granulomatous nephritis and haematuria were observed in immunosuppressed horses with acute kidney injury secondary to nonsteroidal anti-inflammatory (NSAID) administration). Infections by *K. boae* in the kidneys and intestines of constrictor have been associated with haemorrhagic enteritis, intussusception, anorexia and restlessness.

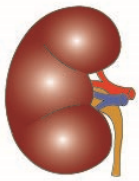
Developmental cycle and mode of transmission: *Klossiella* spp. have homoxenous or monoxenous (one-host) life-cycles involving asexual proliferation (merogony), sexual reproduction (gamogony) and asexual spore formation (sporogony) all occurring within the same host. Infections are transmitted between hosts when mature sporocysts are voided in urine into the external environment where they contaminate food and water supplies to be ingested by new hosts. Transmission does not involve vectors or other alternate intermediate or paratenic hosts. Sporocysts ingested by susceptible hosts excyst in the gut releasing sporozoites that penetrate the gut wall into the circulation. They invade glomerular endothelial cells and form meronts (schizonts) which multiply asexually by internal (endogenous) division forming numerous merozoites. Meronts of some species are occasionally found in capillary endothelia in other organs, either as part of their proliferative cycle (e.g. *K. mabokensis* in intestinal villi) or as ectopic sites of infection. There are often 2 meront generations in the kidneys, with the second generation producing more merozoites than the first (40-100 cf. 8-12). Last generation merozoites invade epithelial cells lining the proximal convoluted tubules and undergo sexual differentiation and reproduction. Female macrogametocytes and male microgametocytes are often found in pairs in tubular epithelial cells and they mature to form single macrogametes and multiple (1-10) nonflagellated microgametes. Fertilization occurs by gamete fusion producing a zygote (sporont) that undergoes multiple fission to form 8-40 sporoblasts and a residual body (it has been argued that these are not true oocyst stages as the surrounding sac appears to be formed by the parasitized host cell rather than laid down by the parasites themselves). Each sporoblast matures into a sporocyst which contains 12-35 sporozoites. These stages greatly distend the host cell until it ruptures releasing sporocysts into the tubule lumen and passing to the bladder to be voided with urine. The prepatent period (time from infection to first sporocyst excretion) was found to vary considerably with reports ranging from 4-5 days, 7-11 days, 30 days and even 6-7 weeks in different hosts. The patent period (duration of sporocyst excretion) was reported to be as long as 8-10 months in some horses. Shed sporocysts are infective to new susceptible hosts when they are ingested via grooming, contact with contaminated fomites, or in contaminated food or water. The sporocysts are not thought to survive for long in the external environment as the thin sporocyst wall would provide little protection against desiccation, heat and solar radiation.

Differential diagnosis: Infections are diagnosed ante-mortem by the microscopic detection of sporocysts in urinary concentrates (sediments, floats), or post-mortem by the detection of developmental stages of histological sections of host tissues. Molecular biological techniques have also been used to characterize isolates following the polymerase chain reaction (PCR) amplification of nuclear (small subunit (18S) ribosomal DNA) and mitochondrial (cytochrome c oxidase I and III, cytochrome b) gene sequences.

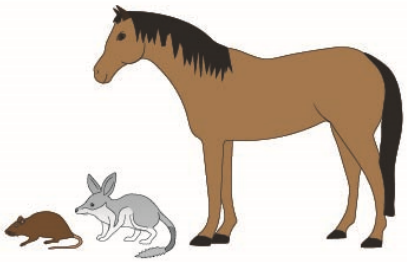
Treatment and control: A small number of drugs have been used in attempts to treat infections in horses, donkeys, marsupials and mice, including sulphonamides alone (sulfadimethoxine) or in combination (sulfadiazine/trimethoprim, co-trimoxazole (trimethoprim/sulfamethoxazole)), coccidiostats (amprolium) and cephalosporin antibiotics (ceftiofur). Few treatments were effective and none appeared to eliminate infections. Animals responded well to supportive care with intravenous fluid therapy, whilst awaiting the restoration of kidney function. Various preventive measures have been implemented in intensive husbandry situations to reduce the risks of urine contamination and parasite transmission, including the regular cleaning and disinfection of animal enclosures, proper waste drainage and elimination, the provision of clean water and food, periodic health screening, isolating and even culling infected individuals.

Klossiella

monoxenous
(1-host)
cycle

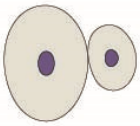


kidney
(tubular nephrosis,
interstitial nephritis,
renal lesions)

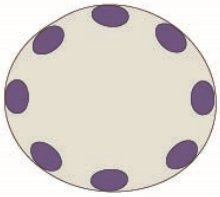


Definitive Hosts
(mammals, esp.
equids, rodents,
marsupials)

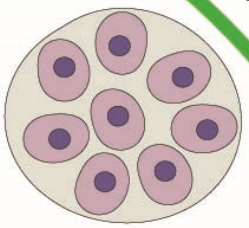
sexual
gamogony



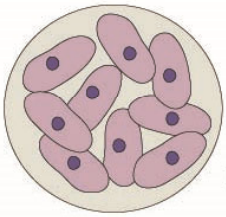
gamonts
(syzygy)
(4-14 µm)



sporont
(10-18 µm)

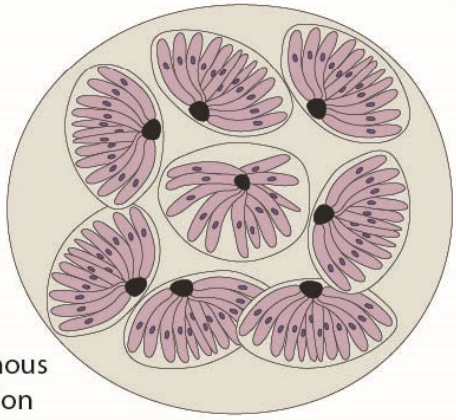


sporoblasts



asexual
merogony

meront
(8-20 µm)



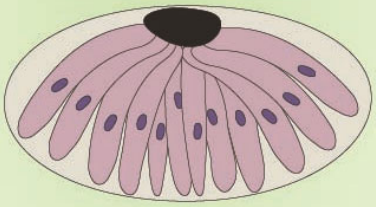
endogenous
sporulation

oocyst
(40-100 µm)

excystation
(sporozoites
released)

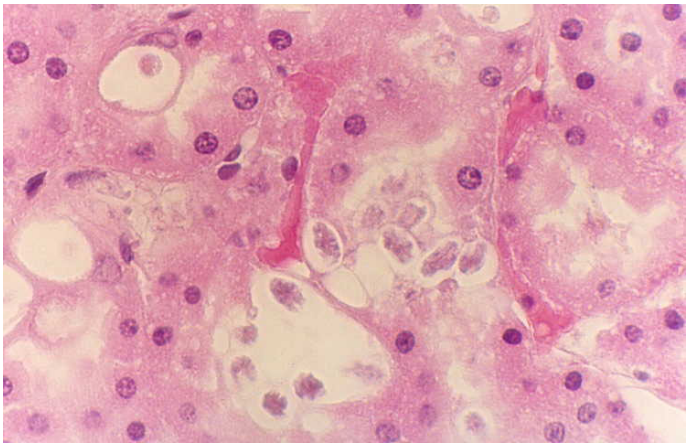
ingested

sporocysts
voided in
urine

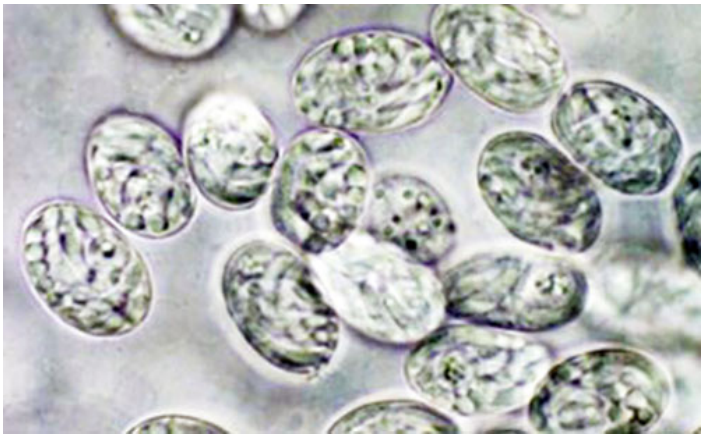


sporocyst
(18-24 µm)

direct transmission via ingestion of sporocysts
contaminating environment (soil, water, food)



Klossiella development in kidney tubules



Klossiella sporocysts in urine