Leucocytozoon
(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. Haematozoa are small blood-borne parasites which undergo merogony (= schizogony) and gamogony (gamete formation) in vertebrates and sporogony (sporozoite formation) in blood-sucking invertebrate vectors. Two main groups are recognised in terrestrial vertebrates: haemosporidia with insect vectors; and piroplasms with arachnid vectors. Haemosporidian parasites multiply in the tissues of vertebrates before forming gamonts in blood cells. Leucocytozoid gamonts occur in leucocytes (sometimes immature erythrocytes) and they do not produce haemozoin pigment. Gametes ingested by insect vectors undergo fertilization in the gut forming motile zygotes (ookinetes) which form oocysts and then thousands of sporozoites which invade the salivary glands. Leucocytozoid infections are transmitted by simuliid black flies and biting midges and are detected in birds, sometimes in association with disease caused by large tissue meronts.

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:	Aconoidasida (asexual stages without conoid)
Order:	Haemosporida (pleomorphic stages in blood of vertebrates, insect vectors. motile zygote (ookinete))
Family:	Leucocytozoidae (schizogony in tissues, gamonts in blood cells, no haemozoin pigment)
Genus:	*Leucocytozoon* (parasitic in tissues/leucocytes of birds)
Species:	various species cause infections in birds
	[*Akiba* effectively *Leucocytozoon caulleryi*]

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: 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: Piroplasmida (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)
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: 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)

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

Haemosporida are spore-forming apicomplexan parasites with heteroxenous life-cycles, with merogony in cells of fixed tissues and in the blood of vertebrate (intermediate) hosts and sporogony in haematophagous invertebrate vectors (definitive hosts). In vertebrate blood cells, haemosporidia develop intracellularly forming sexually dimorphic gametocytes: macrogametocytes (female) with compact nuclei and dark-stained cytoplasm (plentiful ribosomes for protein synthesis), and microgametocytes (male) with larger diffuse nuclei (ready for microgamete production) and pale-staining cytoplasm [a simple pneumonic often used is “blue for girls, pink for boys”]. Gametocytes develop independently (without syzygy) and each microgamont produces about eight flagellated microgametes. Haemozoin granules (residual pigment formed due to incomplete haemoglobin digestion) may or may not be produced in infected erythrocytes. In the vector, the zygote is motile (ookinete) and ultimately forms numerous naked sporozoites (without sporocysts). Around 590 species belonging to some 19 haemosporidian genera have been described from a wide range of mammalian, avian and reptilian hosts around the world.

Four haemosporidian families are recognized mainly on the basis of their developmental cycles and whether haemozoin pigment is produced: namely, Plasmodiidae (merogony in tissues then cyclic in erythrocytes, pigment present); Haemoproteidae (merogony in tissues only, pigment present); Leucocytozoidae (merogony in tissues, pigment absent) and Garniidae (merogony in leucocytes, pigment absent). Vertebrates act as intermediate hosts in which the parasites undergo asexual multiplication within tissues and/or blood cells. Even though parasites begin gamete formation in vertebrates, sexual multiplication is not completed until after they are transmitted to their haematophagous invertebrate vectors, which therefore act as definitive hosts. Two genera are recognized in the family Leucocytozoidae on the basis of multiple biological characters (including morphology, development, host specificity and range): *Leucocytozoon* in birds with simuliid and ceratopogonid vectors; and *Saurocytozoon* in lizards with culicid vectors.

Haemosporidian genera	**No. spp.**	**Site* of development in vertebrate**		**Vertebrate hosts**	**Invertebrate vector**
		meronts	**gamonts**		
Family: Plasmodiidae (merogony in tissues and erythrocytes, haemozoin pigment present)					
Plasmodium	225	liver, rbc	rbc	mammals, birds, reptiles	diptera
Hepatocystis	25	liver	rbc	primates/bats	midges
Polychromophilus	5	viscera	rbc	bats	nycterids
Nycteria	7	liver	rbc	bats	
Biguetiella	1	liver	rbc	bats	
Bioccala	2	RE cells	rbc	bats	
Dionisia	1	liver	rbc	bats	
Rayella	3	liver	rbc	flying squirrels	
Billbraya	1	rbc	rbc	lizards	
Mesnilium	1	RE cells, rbc	rbc	fish	leeches/insects
Haemocystidium (*Simondia*)	33	RE cells	rbc	lizards/tortoises	arthropods
Family: Haemoproteidae (merogony in tissues (not in blood cells), haemozoin pigment present in gametocytes)					
Haemoproteus (*Halteridium*)	6	RE cells	rbc	birds	louse flies
Haemoproteus (*Parahaemoproteus*)	150	RE cells	rbc	birds	midges
Johnsprentia	1	RE cells	rbc	flying foxes	
Sprattiella	1	RE cells	rbc	bats	
Family: Leucocytozoidae (merogony in tissues (not in blood cells), haemozoin pigment absent)					
Leucocytozoon (*Akiba*)	100	RE cells	blood cells	birds	black flies
Saurocytozoon	3	viscera	leucocytes	lizards	mosquitoes
Family: Garniidae (merogony in leucocytes, haemozoin pigment absent)					
Fallisia	12	leucocytes	leucocytes	lizards	arthropods
Garnia	10	leucocytes	leucocytes	lizards	arthropods
Progarnia	1	leucocytes	blood cells	crocodiles	

*rbc = red blood cells (erythrocytes); RE = reticuloendothelial cells

Many molecular phylogenetic studies using nuclear, mitochondrial and apicoplast gene sequences have demonstrated a clear relationship between haemosporidian genera not only with their vertebrate hosts but also their invertebrate vectors. There were clear groupings of *Leucocytozoon* from birds, *Haemoproteus* (*Haemoproteus*) from birds, *Haemoproteus* (*Parahaemoproteus*) from birds, *Plasmodium* from birds and reptiles, *Plasmodium* from rodents and primates, and *Hepatocystis* from bats. These groups were clearly associated with different vectors; namely, simuliids (black-flies), hippoboscids (louse-flies), ceratopogonids (midges), culicine mosquitoes, anopheline mosquitoes, and midges, respectively. There appears to have been a transition from haemosporidia which do not form haemozoin pigment (*Leucocytozoon* in white blood cells) to genera that do form pigment indicating haemoglobin digestion by parasites in red blood cells (*Haemoproteus*, *Plasmodium* and *Hepatocystis*). This was followed by a transition from haemosporidia which undergo schizogony exclusively in host tissues (*Leucocytozoon* and *Haemoproteus*) to those that undergo schizogony in blood cells (*Plasmodium*). While there appears to be a general shift in haemosporidian genera from birds and reptiles to mammals (from nucleated to non-nucleated blood cells), each genus is associated with a particular vector group: black-flies transmitting *Leucocytozoon* to birds; louse-flies transmitting *Haemoproteus* (*Haemoproteus*) to birds; midges transmitting *Haemoproteus* (*Parahaemoproteus*) to birds; culicine mosquitoes transmitting *Plasmodium* to birds and lizards; and anopheline mosquitoes transmitting *Plasmodium* to mammals. The exception to this general trend was *Hepatocystis* which does not undergo blood schizogony and is transmitted to bats by midges.

Around 95 *Leucocytozoon* species have been described in a wide range of bird families and species. They all undergo merogony in internal organs (often forming large meronts divided into cytomeres) before forming gametocytes (lacking pigment) in blood cells (leucocytes and/or erythrocytes). They are transmitted by insect vectors in which they rapidly (3-5 days) form small

nonexpanding oocysts producing < 100 short thick sporozoites. Two *Leucocytozoon* subgenera are recognized: *L.* (*Akiba*) being monotypic with a single species (*L.* (*A.*) *caulleryi*) in galliform birds with biting midge (ceratopogonid) vectors; and *L.* (*Leucocytozoon*) with many species in many bird families with blackfly (simuliid) vectors. Many species appear to have a global distribution, with species richness (biodiversity) being greatest in the Holarctic, Ethiopian and Oriental zoogeographic regions, and poorest in the Australasian and Neotropical regions. Indeed, many infections have been detected in Nearctic migrating birds in their wintering grounds. Host specificity varies from narrow (oioxenous) to broad (stenoxenous), with a few species being host specific (e.g. *L.* (*A.*) *caulleryi* in chickens); some apparently specific for individual bird families or subfamilies (e.g. meleagridids and phasianids in Galliformes, emberizids, icterids and estrildids in Passeriformes) but many thought to be specific for bird orders (esp. Anseriformes, Galliformes, Columbiformes, Charadriiformes, Strigiformes and Passeriformes). Pathogenic *Leucocytozoon* infections have been detected throughout the world, particularly in domestic (ducks, geese, turkeys, chickens) and aviary birds (pigeons, canaries) and occasionally in wild birds (swans, owls, magpies, frogmouths, kestrels).

***Leucocytozoon* species**	**Intermediate hosts (IH)**	**Gametocyte shape (pathogenicity)**	**Definitive hosts (DH) (vectors)**	**Zoogeographic distribution**
Subgenus: *Akiba* (IH: galliform birds, merogony not in hepatocytes; DH: biting midges)				
L. (*A.*) *caulleryi*	Galliformes: phasianid (chicken), numidid (crested guineafowl, helmeted guineafowl)	round (pathogenic, megalomeronts in tissues)	Diptera: ceratopogonid (*Culicoides arakawae, circumscriptus, guttifer, odibilis, schultzei*)	Oriental
Subgenus: *Leucocytozoon* (IH: birds, merogony in hepatocytes; DH: simuliid flies)				
L. (*L.*) *andrewsi*	Galliformes: phasianid (chicken)			cosmopolitan
L. (*L.*) *anellobiae*	Passeriformes: meliphagid (honeyeaters, wattlebirds)	round		Australasian
L. (*L.*) *artamidis*	Passeriformes: artamid (pied butcherbird)	round		Australasian
L. (*L.*) *ardeae*	Pelecaniformes: ardeid (egrets, herons)	round		cosmopolitan
L. (*L.*) *artamidis*	Passeriformes: artamid (pied butcherbird, grey butcherbird, Australian magpie, pied currawong)	round		Australia
L. (*L.*) *audieri*	Accipitriformes: accipitrid (African sea eagle)			African
L. (*L.*) *bacelari*	Accipitriformes: accipitrid (lizard buzzard)			African
L. (*L.*) *balmorali*	Passeriformes: malaconotid (bushshrikes, puffbacks, tchagras), sylviid (warblers)	round, fusiform		Ethiopian
L. (*L.*) *beaurepairei*	Accipitriformes: sagittariid (secretarybird)	fusiform		African
L. (*L.*) *bennetti*	Coraciiformes: coraciid (rollers)	round		Palearctic, African
L. (*L.*) *berestneffi*	Passeriformes: corvid (magpies, jays)	round	Diptera: simuliid (*Simulium aureum, Prosimulium decemarticulatum*)	Holarctic
L. (*L.*) *bonasae*	Galliformes: phasianid (ptarmigans, prairie chicken, prairie grouse, grouse, ruffled grouse, sage grouse, sooty grouse, spruce grouse)			Nearctic
L. (*L.*) *brimonti*	Passeriformes: pyconotid (bulbuls)	round		Oriental, African
L. (*L.*) *bubonis*	Accipitriformes: accipitrid (buzzards, harriers, kites, sparrowhawks); Falconiformes: falconid (falcons)			Holarctic, African
L. (*L.*) *buteonis*	Accipitriformes: accipitrid (common buzzard)	fusiform		Eurasia
L. (*L.*) *californicus*	Falconiformes: falconid (American kestrel)	round		North America

L. (*L.*) *caprimulgi*	Caprimulgiformes: caprimulgid (frogmouths, nightjars)	round, fusiform		Palearctic, Ethiopian, Australasian
L. (*L.*) *centropi*	Cuculiformes: cuculid (coucals, cuckoos)	round		Nearctic, Ethiopian, Oriental
L. (*L.*) *cheissini*	Galliformes: phasianid (snowcocks)	fusiform		Oriental
L. (*L.*) *chloropsidis*	Passeriformes: chloropseid (leafbird)	round		Oriental
L. (*L.*) *circaeti*	Accipitriformes: accipitrid (snake eagle)			Palearctic
L. (*L.*) *coccyzi*	Cuculiformes: cuculid (didric cuckoo, pied cuckoo, yellow-billed cuckoo)			Nearctic
L. (*L.*) *colius*	Coliiformes: coliid (mousebirds)	round		South African
L. (*L.*) *communis* (syn. *bucerotis, dacelo*)	Bucerotiformes: bucerotid (hornbills), upupid (hoopoes, hornbills); Coraciiformes: alcedinid (kingfishers)	round		Ethiopian, Oriental, Australasian, Palearctic
L. (*L.*) *coraciae*	Coraciiformes: coraciid (rollers)			Oriental
L. (*L.*) *costae*	Galliformes: numidid (grey-breasted helmet guineafowl)			African
L. (*L.*) *danilewskyi* (syn. *L. ziemanni*)	Strigiformes: strigid (boobooks, eagle-owls, eared owls, hawk owls, little owls, pygmy owls, scops owls, wood owls), tytonid (barn owls); Accipitriformes: accipitrid (buzzards, eagles, harriers, kites, sparrowhawks); Falconiformes: falconid (falcons); Passeriformes: fringillid (grosbeaks), motacillid (pipits), oriolid (orioles), parid (tits), turdid (blackbirds); Pelecaniformes: ardeid (herons); Bucerotiformes: bucerotid (hornbills); Piciformes: picid (wrynecks); Columbiformes: columbid (doves); Charadriiformes: glareolid (waders)	round, fusiform (megalomeronts in organs)	Diptera: simuliid (*Prosimulium decemarticulatus, Simulium aureum, latipes, vernum*)	cosmopolitan (except Antarctic)
L. (*L.*) *dinizi*	Musophagiformes: musophagid (plaintain-eaters, turacos)	round		Ethiopian
L. (*L.*) *dubreuili* (syn. *francai, nectariniae, seabrae*)	Passeriformes: bombycillid (waxwings), fringillid (finches, grosbeaks), icterid (cowbirds), mimid (catbirds), muscicapid (chats, flycatchers, redstarts, warblers), parid (tits), parulid (ovenbirds), passerid (sparrows), pycnonotid (bulbuls), sturnid (starlings), turdid (bluebirds, robins, robin-chats, thrushes)	round	Diptera: simuliid (*Prosimulium decemarticulatum, Cnephia ornithophila, Simulium aureum, latipes, quebecense, vernum*)	cosmopolitan (except Antarctic)
L. (*L.*) *enriquesi*	Passeriformes: chloropseid (blue-winged leafbird)			Oriental
L. (*L.*) *eurystomi* (syn. *huchzermeyeri*)	Coraciiformes: coraciid (kingfishers, rollers), meropid (bee-eaters)	round, fusiform		Palearctic, Ethiopian, Oriental
L. (*L.*) *francai*	Coraciiformes: coraciid (broad-billed roller)			African
L. (*L.*) *franchini*	Passeriformes: corvid (blue jays, Eurasian jays); Galliformes: phasianid (Barbary partridge)			Holarctic, African
L. (*L.*) *frasci*	Coraciiformes: coraciid (ground rollers)	round		African
L. (*L.*) *fringillinarum* (syn. *bouffardi, cambournaci, deswardti, dutoiti, gentili, icteris, monardi,*	Passeriformes: alaudid (larks), bombycillid (waxwings), cardinalid (cardinals), certhiid (treecreepers), cisticolid (cisticolas, prinias), corvid (jays), emberizid (buntings), estrildid (mannikins, munias, pytilias), fringillid (finches, crossbills, grosbeaks, redpolls),	round	Diptera: simuliid (*Cnephia ornithophilia, Prosimulium (Helodon) decemarticulatus, Simulium*	cosmopolitan (except Antarctic)

muscicapa, parulis, pittae p.p., prionopis, roubaudi, sturni, thraupis, timallae, whitworthi)	glareolid (warblers), hirudinid (swallows), icterid (cowbirds, grackles), laniid (shrikes), malaconotid (boubous), melanocharitid (longbills), meliphagid (friarbirds, honeyeaters, miners, wattlebirds), mimid (catbirds), motacillid (pipits, wagtails), muscicapid (flycatchers, nightingales), nectariniid (sunbirds), oriolid (orioles), paradisaeid (birds-of-paradise), parid (tits), parulid (ovenbirds), passerellid (juncos, towhees), passerid (sparrows), ploceid (weavers, fodies), pycnonotid (bulbuls), sturnid (starlings), thraupid (tanagers), turdid (blackbirds, robins, thrushes), viduid (indigobirds), vireonid (vireos); Musophagiformes: musophagid (turacos); Piciformes: capitonid (barbets)		*anatinum, annulus, aureum, latipes, quebecense*, *vernum, venustum*)	
L. (*L.*) *giovannolai*	Passeriformes: turdid (redwings)			Palearctic
L. (*L.*) *grallariae*	Passeriformes: grallariid (undulated antpitta)	fusiform		South America
L. (*L.*) *greineri*	Passeriformes: nectariniid (sunbirds), philepittid (velvet asity)	round		African
L. (*L.*) *grusi*	Gruiformes: gruid (cranes)	round, fusiform		Nearctic
L. (*L.*) *hamiltoni*	Passeriformes: parid (tits)	symmetrical fusiform		Palearctic
L. (*L.*) *hirundinis*	Passeriformes: hirudinid (swallows)			Holarctic
L. (*L.*) *ibisi*	Pelecaniformes: threskiornithid (white ibis)	round		Australasian
L. (*L.*) *irenis*	Passeriformes: irenid (fairy bluebirds)	round		Oriental
L. (*L.*) *kerandeli*	Galliformes: phasianid (francolins)			African
L. (*L.*) *lairdi*	Passeriformes: vangid (vangas)	round		African
L. (*L.*) *leboeufi* (syn. *ardeolae, iowense*)	Pelecaniformes: ardeid (bitterns, herons, ibis), Ciconiiformes: ciconiid (storks)	round		Holarctic, Ethiopian, Oriental
L. (*L.*) *legeri*	Charadriiformes: scolopacid (snipes, stints, waders, woodcocks)	round		Palearctic
L. (*L.*) *liothricis*	Passeriformes: leiothricid (red-billed leiothrix)	round		Oriental
L. (*L.*) *lovati*	Galliformes: phasianid (grouse)	round, fusiform	Diptera: simuliid (*Simulium aureum, croxtoni, latipes, quebecense, vernum*)	Holarctic
L. (*L.*) *lutzi*	Strigiformes: strigid (pygmy owls)			Neotropical
L. (*L.*) *maccluri*	Passeriformes: turdid (darksided thrush)	round, fusiform		Oriental
L. (*L.*) *macleani* (syn. *francolini*)	Galliformes: phasianid (partridges, peafowl, pheasant, quail, chickens)	round, fusiform (pathogenic in chickens)	Diptera: simuliid (*Simulium metatarsale*)	Palearctic, Ethiopian, Oriental
L. (*L.*) *majoris* [type species] (syn. *bishopi, oriolis, pitae p.p., pycnonoti*)	Passeriformes: corvid (jays), fringillid (finches), glareolid (warblers), laniid (shrikes), mimid (catbirds), nectariniid (sunbirds), parid (tits), parulid (ovenbirds, yellowthroats), passerid (sparrows), pycnonotid (bulbuls), sturnid (starlings), thraupid (tanagers), turdid (blackbirds, thrushes)	round		cosmopolitan (except Neotropical, Antarctic)
L. (*L.*) *mansoni*	Galliformes: phasianid (wood grouse)			Palearctic
L. (*L.*) *marchouxi*	Columbiformes: columbid (doves, pigeons)	round		Holarctic, Ethiopian, Oriental, Neotropical

L. (*L.*) *martini*	Galliformes: phasianid (Oriental peafowl)			Oriental
L. (*L.*) *martyi*	Accipitriformes: accipitrid (Indian goshawk)			Oriental
L. (*L.*) *mathisi*	Accipitriformes: accipitrid (buzzards, sparrowhawks)	fusiform		cosmopolitan
L. (*L.*) *melloi*	Coraciiformes: coraciid (Indian roller)			Oriental
L. (*L.*) *mesnili*	Galliformes: phasianid (francolins, partridges, pheasant, quail); Pterocliformes: pteroclid (sandgrouse); Otidiformes: otidid (bustards); Columbiformes: columbid (doves)			Palearctic, African, Oriental
L. (*L.*) *mirandae*	Passeriformes: corvid (jays), turdid (thrushes); Galliformes: phasianid (grouse)			Palearctic, African, Oriental
L. (*L.*) *molpastis*	Passeriformes: pycnonotid (bulbuls)			Oriental
L. (*L.*) *muratovi*	Accipitriformes: accipitrid (marsh harrier)			African
L. (*L.*) *neavei*	Galliformes: phasianid (francolins, pheasant), numidid (guineafowl); Strigiformes: strigid (eagle-owls, owlets); Accipitriformes: accipitrid (kites)	round, fusiform	Diptera: simuliid (*Simulium adersi, nyasalandicum*)	Ethiopian
L. (*L.*) *neophrontis*	Accipitriformes: accipitrid (vultures)			African
L. (*L.*) *neotropicalis*	Passeriformes: cotingid (green-and-black fruiteater)	fusiform		South America
L. (*L.*) *nycticoraxi*	Pelecaniformes: ardeid (herons)	round, fusiform		Palearctic
L. (*L.*) *nyctyornis* (syn. *alcedinis*)	Coraciiformes: alcedinid (kingfishers), meropid (bee-eaters)	round		Palearctic, Ethiopian, Oriental
L. (*L.*) *otidis*	Otidiformes: otidid (Kori bustard)	round		African
L. (*L.*) *peaolopesi*	Galliformes: phasianid (red-necked spurfowl)	round-fusiform		African
L. (*L.*) *peircei*	Passeriformes: glareolid (warblers)	fusiform		African
L. (*L.*) *phylloscopus*	Passeriformes: phylloscopid (greenish warbler)	round		Palearctic
L. (*L.*) *podargii*	Caprimulgiformes: caprimulgid (tawny frogmouth)	round		Australasian
L. (*L.*) *polynuclearis*	Piciformes: picid (northern flicker, white-headed woodpecker)	round		North America
L. (*L.*) *pogoniuli*	Piciformes: capitonid (barbets)	round		African
L. (*L.*) *pterotenuis*	Passeriformes: grallariid (chestnut-crowned antpitta)	fusiform		South America
L. (*L.*) *quynzae*	Apodiformes: trochilid (amethyst-throated sunangel, mountain avocetbill, Tyrian metaltail, blue-throated starfrontlet)	round		South America
L. (*L.*) *ralli species inquirenda*	Gruiformes: rallid (water rails)	round		Palearctic, African
L. (*L.*) *sabrazesi*	Galliformes: phasianid (chickens, francolins, pheasant)			Oriental, African
L. (*L.*) *sakharoffi* (syn. *laverni*)	Passeriformes: corvid (ravens, treepies, jays, nutcrackers)	round (megalomeronts in tissues)	Diptera: simuliid (*Simulium angustitarse, aureum, latipes, Prosimulium decemarticulatum*)	Holarctic
L. (*L.*) *sanarelli*	Pelecaniformes: ardeid (night herons)	round		Australasian
L. (*L.*) *schoutedeni*	Galliformes: phasianid (chickens)	round (low pathogenicity)	Diptera: simuliid (*Simulium adersi, impukane, nyasalandicum,*	Ethiopian, Nearctic, Oriental

			vorax)	
L. (*L.*) *shaartusicum*	Passeriformes: muscicapid (African stonechats)	round		African
L. (*L.*) *simondi*	Anseriformes: anatid (ducks, eiders, geese, mergansers, swans)	round, fusiform (pathogenic, esp. in young birds, due to anaemia and organ damage)	Diptera: simuliid (*Cnephia ornithophila, Simulium anatinum, fallisi, innocens, parnassum, rendalense, rugglesi, usovae, venustum, vittatum*)	Holarctic
L. (*L.*) *smithi*	Galliformes: phasianid (turkeys, partridges)	round, fusiform (pathogenic in turkeys due to organ congestion	Diptera: simuliid (*Prosimulium hirtipes, Simulium aureum, congareenarum, jenningsi, meridionale, pictipes, slossonae, vittatum*)	Holarctic, Ethiopian
L. (*L.*) *sousadiasi*	Charadriiformes: charadriid (waders)	fusiform		Ethiopian
L. (*L.*) *squamatus* (syn. *capitonis*)	Piciformes: megalaimid (barbets), picid (woodpeckers, wrynecks)	round		Holarctic, Ethiopian, Oriental
L. (*L.*) *struthionis*	Struthioniformes: struthionid (ostriches)	round (pathogenic in chicks)		Ethiopian
L. (*L.*) *tawaki*	Sphenisciformes: spheniscid (penguins)	round	Diptera: simuliid (*Austrosimulium australense, dumbletoni, ungulatum*)	New Zealand, South African
L. (*L.*) *toddi*	Accipitriformes: accipitrid (black eagle, buzzards, harriers, kites, sparrowhawks, vultures); Falconiformes: falconid (eagles, falcons, hawks)	round, fusiform (pathogenic in chicks)	Diptera: simuliid (*Simulium aureum, quebecense, vernum, Prosimulium decemarticulatum*)	Holarctic, Neotropical, Ethiopian, Oriental
L. (*L.*) *trachyphoni*	Piciformes: megalaimid (barbets)	round		African
L. (*L.*) *underhilli*	Passeriformes: sturnid (starlings)	round		African
L. (*L.*) *vandenbrandeni*	Suliformes: sulid (darters); Pelecaniformes: phalacrocoracid (cormorants)	round		Ethiopian, Australasian
L. (*L.*) *vangis*	Passeriformes: vangid (hook-billed vanga)	round		African
L. (*L.*) *zosteropis*	Passeriformes: zosteropid (white-eyes)	round		African, Oriental

A small number of *Saurocytozoon* spp. infect reptiles (lizards) forming meronts in tissues (in lymphocytes?) before forming large gametocytes (without pigment) in peripheral blood (leucocytes) causing gross host cell distortion. They are transmitted by culicine mosquitoes in which large expanding oocysts develop slowly (over 16 days) producing several hundred long thin sporozoites.

***Saurocytozoon* species**	**Intermediate hosts**	**Gametocyte shape**	**Definitive hosts (vectors)**	**Geographic distribution**
S. agamidorum	Sauria: agamid (rock agama)	ovoid		southern Asia
S. mabuyi	Sauria: scincid (sun skinks)	ovoid		South America
S. tupinambi [type species]	Sauria: teiid (gold tegu)	ovoid	Diptera: culicid (*Culex pipiens*)	South America

Parasite morphology: *Leucocytozoon* spp. form 2 main developmental stages in birds (tissue meronts and blood gametocytes) and 4 stages in insect vectors (gametes fusing to form ookinetes that mature to oocysts producing sporozoites). First generation exo-erythrocytic meronts appear as rounded basophilic bodies (10-45 μm) that often invaginate and break into separate parts (cytomeres, or spawning syncytia) containing numerous uninuclear merozoites (1-2 μm). These merozoites may invade blood cells where they transform into small rounded gametocytes (2-5 μm) with cup-like peripheral nuclei (the parasites do not undergo erythrocytic merogony). Merozoites or syncytia (meront fragments with 2 or more nuclei) are also spread via the blood to various organs where they are phagocytosed by macrophages and other reticuloendothelial cells. They may then give rise to large megalomeronts (50-400 μm) which are host cell-parasite complexes with a central body (markedly hypertrophied host cell nucleus) and numerous cytomeres that produce thousands of small merozoites (1 μm). Merozoites from megalomeronts may invade other reticuloendothelial cells and repeat the process (thus contributing to chronic infections and sometimes spring relapses) or they may invade blood cells where they transform into round-fusiform gametocytes (12-22 μm) that displace the host cell nuclei laterally and often cause the host cells to become elongate and spindle-shaped. Some parasite species form round gametocytes, others form fusiform gametocytes, and several form both. None contain haemozoin pigment, but some may have volutin (pseudo-pigment) granules. Gametocytes exhibit sexual dimorphism, with macrogametocytes (female) having a dark-staining cytoplasm (due to abundant ribosomes) and a compact nucleus, while microgametocytes (male) have a pale-staining cytoplasm and a larger diffuse nucleus. In vectors, microgamonts exflagellate producing several (up to 8) long thin (20-23 μm) microgametes that fertilize more robust uninucleate macrogametes. The resultant zygote (known as an ookinete) is motile and is elongate and saccular (30 x 4 μm) with a central nucleus and numerous clear cytoplasmic vacuoles. The ookinete develops into a spherical oocyst (9-14 μm) surrounded by capsule-like wall. The oocyst produces numerous (< 100) elongate sporozoites (8 x 1 μm) with one pointed end and the other rounded.

Site of infection: In birds, sporozoites invade hepatocytes in the liver and undergo merogonous proliferation. The parasites then invade immature blood cells (erythrocytes or leucocytes) and develop into gametocytes, or they invade macrophages in host tissues (spleen, lymph nodes, brain, heart, liver, kidney) to form large megaloschizonts. Almost 95 parasite species have been recorded in birds belonging to 23 orders (from 42 passerine families and 33 non-passerine families). Many parasite species appear to have a global distribution, with species richness (biodiversity) being greatest in the Holarctic, Ethiopian and Oriental zoogeographic regions, and poorest in the Australasian and Neotropical regions. The parasite species vary in their host specificity from narrow (oioxenous) to broad (stenoxenous), with a few species being specific for individual host species, some being specific for individual bird families or subfamilies, but many apparently being specific for bird orders. In insect vectors, gametes are formed in the gut, ookinete zygotes move through the gut wall to form oocysts near the basal lamina, and sporozoites are liberated into the haemocoel where they invade the salivary glands. Vectors are known only for some 14 *Leucocytozoon* spp., all using with simuliid blackflies (totaling 34 species in 4 genera, mostly *Simulium* but also some *Austrosimulium, Cnephia* and *Prosimulium* spp.) and the sole exception being *L.* (*Akiba*) *caulleryi* which uses ceratopogonid biting midges (5 species in the genus *Culicoides*).

Pathogenesis: Infections in birds are characterized by the merogonous proliferation of parasites in host tissues leading to the carriage of gametocytes in blood cells. Tissue stages are transient but ultimately lyse host cells sometimes causing disruptive lesions with variable inflammation, while blood stages persist as transmissive stages until infected cells are removed or degenerate. The development of tissue meronts is often asynchronous, with different stages linked to variable rates of tissue penetration and multiplication. Most infections are asymptomatic or subclinical and have not been associated with clinical disease. However, the occurrence of multiple meronts (especially megalomeronts) may cause mild-severe disease (leucocytozoonosis) characterized by small necrotic or large space-occupying lesions, impedance of blood flow, ischaemia, focal haemorrhages due to burst capillaries (disease sometimes called haemorrhagic fever), inflammation with cellular infiltrates (macrophages, plasma cells, heterophils) and eventual fibrosis and calcification. Clinical signs exhibited by birds vary depending on the tissues/organs involved, but have included anaemia, leucocytosis, hepatosplenomegaly (but no pigment accumulation), haemorrhages, anaemia, respiratory difficulties, diarrhoea, myositis, listlessness, lethargy, anorexia, emaciation, debilitation, convulsions, paralysis, and death. In contrast, blood stages have rarely been associated with pathogenic changes although they may contribute to anaemic conditions, less due to haemolysis but more due to the erythrophagocytosis of infected cells by reticuloendothelial cells in the haemopoetic system.

Anaemia may also be exacerbated by the phagocytosis of uninfected cells due to the appearance of an anti-erythrocyte factor in plasma which increases their osmotic fragility with subsequent haemolysis. Several parasite species are highly pathogenic in domestic poultry and waterfowl with outbreaks of disease causing significant economic losses in chicken, turkey, goose and duck production. Occasional epizootics have been reported in free-range wild birds, including illness and deaths in swans, geese, ducks and weaver finches. There are few outbreaks in zoo or aviary birds possibly due to the lower abundance of natural vectors, but some clinical cases have been associated with translocations of exotic bird species. In general, infections may occur in birds of all ages: young birds are more susceptible but older birds have more sustained exposure to vectors. Studies have suggested that even subclinical infections in young birds may affect their productivity (reduced growth rates, fewer eggs) and fitness (reduced mating intensity, and delayed migration). Several studies have also indicated that infections in simuliid blackflies may reduce vector fitness and competence as evidenced by shorter lifespans. Bird species at greatest risk of infection are those with long nesting periods, open or ground nests, close proximity to aquatic breeding sites for blackfly vectors, and gregarious or flocking behaviours. While infections may last months following single challenge, persistent exo-erythrocytic tissue stages may lead to intermittent relapses of parasitaemia and the development of chronic infections, but at the same time may provide some protection from disease through the development of premunitive immunity. In countries with temperate climates, relapses tend to occur in the spring and have tentatively been associated host sex hormone changes at the onset of breeding.

Developmental cycle and mode of transmission: *Leucocytozoon* spp. have obligate heteroxenous (2-host) life-cycles with transmission occurring between vertebrate intermediate hosts (birds) and invertebrate definitive hosts (haematophagous dipteran insects). Transmission occurs via vector bite during blood feeding. Most species utilize blackflies (simuliids) as vectors, while one species uses biting midges (ceratopogonids). Sporozoites injected into birds by blood-feeding vectors invade hepatocytes and form first-generation exo-erythrocytic meronts over 4-5 days. The meronts undergo asexual internal division forming separate cytomeres (spawning syncytia) that produce numerous merozoites which are released into the circulation when the mature meronts bursts the host cell. The merozoites may invade other hepatocytes and undergo further merogonous division, or they may invade red blood cells (erythroblasts and erythrocytes) and form small rounded gametocytes over 5 days (no merogony in blood cells). In some parasite species, merozoites or syncytia (meront fragments with 2 or more nuclei) may infect macrophages and reticuloendothelial cells in host tissues forming large exo-erythrocytic megalomeronts which develop slowly to produce thousands of merozoites. These merozoites may periodically lead to the development of more megalomeronts (contributing to spring relapses and chronic infections) or they may invade white blood cells (lymphocytes and other mononuclear leucocytes) and form large fusiform gametocytes. Several parasite species have been observed to exhibit diurnal cycles of parasitaemia (peaking during the daytime). Infections are transmitted to simuliid black flies when they ingest blood cells containing gametocytes when feeding on bird blood. Gametocytes complete gamete formation in the gut with microgametes fertilizing macrogametes over 5 days to produce motile zygotes. These ookinetes penetrate the gut peritrophic membrane and form oocysts near the basal lamina without bulging into the haemocoel. The oocysts mature over 7-18 days and produce numerous sporozoites (< 100) that are released into the haemocoel where they invade the salivary glands or enter the proboscis directly. Sporozoites are injected into birds when the vector feeds on blood (inoculative transmission). Studies have noted differences in vector competence and specificity depending on their feeding specializations (mammals or birds as preferred hosts), vertical distribution (ground-dwelling or tree canopy), temporal abundance (seasonal or year-round) and prevailing climatic conditions (warm weather in high latitudes, or humid periods following rains). It is noteworthy that many infections have been detected in Nearctic migrating birds in their wintering grounds.

Differential diagnosis: Infections are diagnosed by the direct microscopic detection of gametocytes in blood cells in thin blood smears stained with any of the Romanowsky's stains, notably Giemsa. It is recommended that multiple serial blood samples be examined to detect low or intermittent parasitaemias. Tissue meronts or megalomeronts may be detected following autopsy by the microscopic examination of tissue impression smears or histological sections. The identification of individual parasite species is difficult due to the pleomorphy demonstrated by many stages and their ready deformation during processing, the high over-lapping inter-specific variability in many characters, and differences in their developmental cycles (only some form megalomeronts, and they may form fusiform or rounded gametocytes, or both). Vectors may also be trapped and examined for infections by oocysts and sporozoites by dissection and microscopic examination of gut or salivary gland preparations. Several immunoserological tests (latex agglutination, enzyme immunoassays) have been used experimentally to monitor specific antibody responses in birds, but little is known about their sensitivity or specificity. Molecular biological techniques have been used to detect and characterize parasites by the polymerase chain reaction (PCR) amplification of nuclear (small subunit (18S) ribosomal DNA) and mitochondrial (cytochrome b) gene sequences.

Treatment and control: Most clinical infections are detected after considerable damage has been wrought by parasite proliferative stages in vital organs of the host, so chemotherapeutic (curative) treatments have not been very successful. Several antiprotozoal (antimalarial and coccidiostatic) drugs have been found to be effective in reducing rates of infection, parasitaemia and disease when used for chemoprophylactic (preventive) purposes in poultry houses or aviaries as food or water additives, including sulphonamides, pyrimethamine, trimethoprim-sulfamethoxazole, sulphadimethoxine, furazolidone, quinacrine and clopidol. Other preventive measures have centred around vector control by protecting birds using insect screens and topical or environmental insecticides. Experimental vaccination studies have reported good protection in chickens immunized with recombinant R7 protein from *L.* (*Akiba*) *caulleryi* which elicited strong humoral antibody responses.

Leucocytozoon

2 subgenera:
L. (*Akiba*) in galliform birds with ceratopogonid vectors
L. (*Leucocytozoon*) in many bird families with simuliid blackfly vectors

heteroxenous (2-host) cycle
vector-borne transmission
(sexual development in invertebrate host)
(asexual development in vertebrate host)

Definitive Hosts (vectors)
(blackflies, biting midges)

oocyst
(9-14 µm)

sporozoites
(~ 8 µm)

gut

ookinete
(30 µm)

fertilization

gametes

salivary gland

gametocytes ingested

sporozoites injected

male female

erythrocytes

round gametocytes
(2-5 µm)

meront
(10-45 µm)

liver merogony
(hepatocytes)

trophozoite
(1-2 µm)

male female

leucocytes

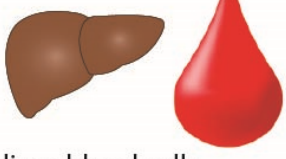
fusiform gametocytes
(10-22 µm)

megalomeront
(50-400 µm)
(internal tissues)

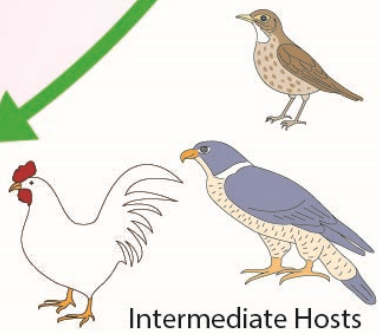
tissue merogony
(macrophages)

syncytia
(1-5 µm)

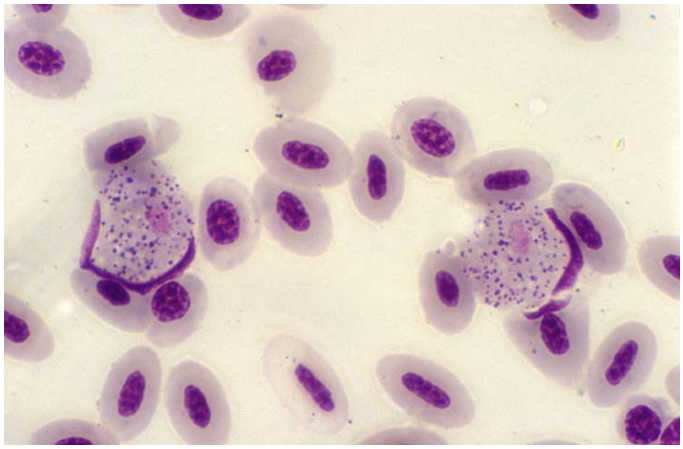
meronts often divided internally into cytomeres



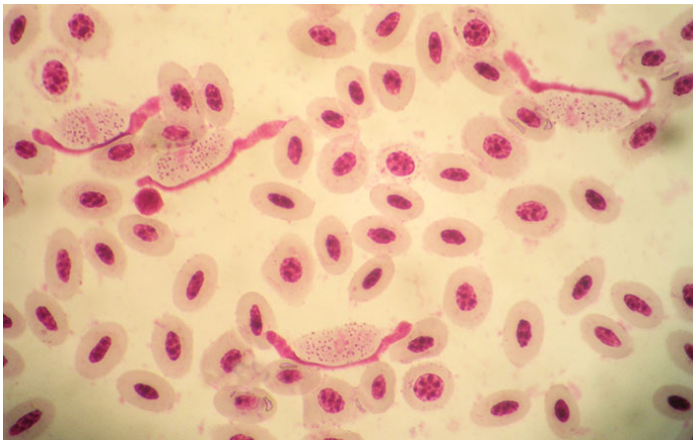
liver, blood cells
(sometimes necrotic lesions, ischaemia)



Intermediate Hosts
(birds)



Leucocytozoon round gametocytes in bird blood



Leucocytozoon fusiform gametocytes in bird blood

Leucocytozoon megalomeront in bird tissues