Neospora
(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. *Neospora* spp. only form oocysts in dogs – merogony (= schizogony), gamogony (male microgametes fertilize female macrogametes) and oocyst formation occur in the intestinal epithelia. Sporogony occurs exogenously and mature oocysts contain 2 sporocysts each with 4 sporozoites (1:2:4 configuration). Thick-walled tissue cysts filled with bradyzoites (= cystozoites) are formed in the central nervous systems of domestic ruminants, horses and dogs following merogonous division of tachyzoites in macrophages. Enteric infections in dogs appear to be asymptomatic but systemic infections may produce neurological lesions in dogs as well as abortion and neonatal mortalities in ruminants, especially cattle.

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:	*Neospora* (tissue cyst-forming coccidian parasites of mammals)
Species:	several species cause neosporosis in ruminants, equids and canids

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 *Neospora* was described in the 1980s as the aetiological agent of a unique neurological disease involving hindlimb paralysis in dogs, while subsequent studies found it to be a major cause of abortion in both beef and dairy cattle around the world. The parasite was similar to *Toxoplasma*, but *Neospora* tissue cysts had thicker walls (1–4 cf. 0.5 μm), were more restricted to neural tissue (cf. many tissues for *Toxoplasma*), and were antigenically distinct (exhibiting little cross-reactivity with *Toxoplasma*). Infections have been reported in a wide range of intermediate hosts, especially carnivores (12 families), several ungulates (bovids, cervids and equids), a few birds (passerids, corvids and accipitrids), and experimentally to rodents. Experimental transmission studies found the definitive hosts for *Neospora* to be canids (dogs, wolves, foxes) rather than felids (as occurs for *Toxoplasma*). It appears that all life-cycle stages; namely, tachyzoites (in meronts), bradyzoites (in tissue cysts), and sporozoites (in oocysts) are infectious for both intermediate and definitive hosts, and transmission has been confirmed by oocyst ingestion (faecal-oral transmission), tissue cyst ingestion (predator-prey transmission) and transplacental passage (vertical transmission). Molecular characterization studies using multiple gene sequences have identified 2 *Neospora* spp.; *N. caninum* in dogs, cattle, sheep, goats, horses and deer in North America, Europe and Australia; and *N. hughesi* in horses with neurological disease in North America.

Neospora species	Intermediate hosts (IH)	Definitive hosts	Distribution
N. caninum	Carnivora: canid (dog), ursid (brown bear), hyaenid (spotted hyena), mustelid (fisher, Eurasian badger, pine martin, European polecat, ferret, ermine, beech marten, American mink, Eurasian otter, North American river otter, sea otter), herpestid (Egyptian mongoose), viverrid (common genet), ailurid (red panda), procyonid (raccoon), felid (cat, wildcat, cheetah, caracal, colocolo, Geoffroy's cat, ocelot, little spotted cat, serval, lynx, fishing cat, puma, jaguar, jaguarundi, leopard, clouded leopard, snow leopard, tiger, lion), otariid (sea lion), phocid (bearded seal, harbor seal, ribbon seal, ringed seal, spotted seal), odobenid (walrus); Artiodactyla: bovid (cattle, buffalo, sheep, goat), cervid (deer, whitetail), Perissodactyla: equid (horse), Galliformes: phasianid (chicken), Passeriformes: corvid (magpie), passerid (house sparrow): Accipitriformes: accipitrid (buzzards); plus experimental infection in Rodentia: murid (rat, mouse, gerbil), Lagomorpha: leporid (rabbit), Artiodactyla: suid (pig), Carnivora: canid (dingo, fox); also diheteroxenous (IH-IH) vertical (transplacental) transmission.	Carnivora: canid (dog, dingo, bush dog, coyote, culpeo, red fox, gray fox, crab-eating fox, fennec fox, Pampas fox, hoary fox, blue fox, Chiloe fox, maned wolf, Eurasian wolf, gray wolf, golden jackal, raccoon dog)	worldwide
N. hughesi	Perissodactyla: equid (horse) [cause of equine protozoal myeloencephalitis (EPM)]	unknown	North America

Parasite morphology: *Neospora* spp. form 4 different types of developmental stages: meronts producing tachyzoites, cysts producing bradyzoites, gamonts producing gametes, and oocysts producing sporocysts and sporozoites. Meronts (sometimes called schizonts) are asexual proliferative forms located within parasitophorous vacuoles in a range of cells in intermediate hosts. The appear as rounded basophilic bodies (10-20 µm) that undergo rapid internal division (repeated endodyogeny) to form numerous (4-20) merozoites (usually called tachyzoites – 'tachy' meaning 'fast'). Tachyzoites may be ovoid/globular, rod-like, lunate/crescentic or banana-shaped ranging from 4.3-8.4 x 1.0-2.5 (often 6 x 2) µm depending on their state of maturation. They may be located within a parasitophorous vacuole or lie free in the host cell cytoplasm. Ultrastructurally, each tachyzoite possessed an anterior conoid, 4-24 rhoptries, micronemes, dense granules, a single nucleus and was bound by a pellicular membrane with 22 subpellicular microtubules. Tissue cysts are encapsulated stages found within host cells throughout the body. Cysts are microscopic but vary in size (6-107 µm long) depending on their age, tissue tropism and host specificity. They are aseptate and bound by a PAS-positive (periodic acid-Schiffs reagent) primary cyst wall (0.5-4.0 µm thick) consisting of a thickened unit membrane that may have a wavy contour but lacks protrusions and a secondary cyst wall. *Neospora* cysts are morphologically similar to those of *Toxoplasma* (although the cyst wall of the latter is thinner at 0.5 µm), but they are serologically distinct and do not cross-react with *Toxoplasma* immune sera or antisera. Internally, the cysts form cystozoites (usually called bradyzoites – 'brady' meaning 'slow) that multiply by internal division (endodyogeny) such that larger cysts may contain 200 or more. Bradyzoites are elongate cells (4-8 x 1-3 µm) that contain a conoid, 6-12 rhoptries, micronemes, dense granules, a subterminal nucleus and pellicular membranes with 22 subpellicular microtubules (some studies suggest that bradyzoites can be differentiated from tachyzoites by certain subcellular features, but several reviews have revealed considerable pleomorphy between isolates). Parasite development in the intestines of definitive hosts is known to culminate in the production of characteristic oocysts, but the preceding asexual and/or sexual developmental stages have yet to be demonstrated. One study tentatively identified schizont-like structures (basophilic bodies 8-12 µm) that labelled strongly with *Neospora* antisera, as well as weakly-labelled microgamont-like bodies ('small spots with flagella-like structures'). Developing oocysts have been found in mucosal and crypt epithelial cells as round-oval stages (around 10 µm in diameter) that are bound by smooth membranous oocyst walls (0.6–0.8 µm thick). Mature oocysts (9.4-13.4 µm) are shed unsporulated in host faeces and then undergo internal division (sporulation) to form 2 sporocysts (7.0-9.0 x 5.6-6.4 µm), each of which forms 4 elongate sporozoites (6.0-7.0 x 1.8-2.2 µm) and a residual body. The oocysts therefore have an isosporid configuration (1:2:4 oocyst:sporocyst:sporozoite) and are morphologically similar to those of *Toxoplasma* and *Hammondia.*

Site of infection: Most *Neospora* infections have involved the detection of tissue cysts in intermediate host tissues, particularly in the brain, spinal cord and skeletal muscles, but sometimes in hepatic, uterine, placental and foetal tissues (often in vascular endothelial cells and macrophages). Developing meronts (schizonts) have also been found in many cell types, including nerve and

muscle cells, hepatocytes, macrophages, renal tubular epithelial cells, skin cells, fibroblasts, vascular endothelial cells, and even retinal cells. Infections by *N. caninum* have been recorded in around 60 vertebrate species: mostly mammals, including 7 artiodactylans (bovid, cervid, suid), one perissodactylan (equid), 44 carnivores (ailurid, canid, felid, herpestid, hyaenid, mustelid, odobenid, otariid, phocid, procyonid, ursid, viverrid), 3 rodents (murid), and one lagomorph (leporid); and 4 bird species (phasianid, corvid, passerid, and accipitrid). In contrast, infections by *N. hughesi* have only been detected in the neural tissues of horses. Following the discovery that *N. caninum* was able to undergo sexual reproduction in carnivorous (definitive) hosts, oocyst production has been documented in some 18 canid species (dogs, wolves, foxes, jackals), but the detection of earlier schizont and gamont developmental stages has remained elusive. One study reported the occurrence of schizont-like and microgamont-like stages in intestinal epithelial cells, while several studies have demonstrated developing oocysts in jejunal and ileal epithelia.

Pathogenesis: The intracellular (schizogonous) proliferation of parasites ultimately leads to host cell lysis releasing tachyzoites to invade other cells. Their subsequent encystment in host tissues and the formation of cysts containing numerous bradyzoites results in small space-occupying lesions often associated with inflammation, necrosis and fibrosis. Light infections may remain asymptomatic or subclinical, but heavier disseminated infections may cause mild-severe clinical disease (neosporosis). Infections in dogs may take 2 forms depending on whether they act as definitive or intermediate hosts: enteric infections by developing oocysts have not been associated with disease; while systemic infections by schizonts and cysts may cause neuromuscular degeneration leading to ascending paralysis of the limbs. Hyperextension is common due to myositis and upper motor neuron paralysis leading to fibrous contracture of muscles and fixation of joints (arthrogryposis). Infected animals may die at any age, but most mortalities occur in young dogs (< 6 months old). Infections may also cause abortion in pregnant dogs, but more commonly they give birth to infected pups which may exhibit muscle atrophy and ascending paralysis. Infections in cattle are generally latent and asymptomatic in non-pregnant animals, but primary, secondary or recrudescent infections in pregnant cows can result in mid-gestation abortions, foetal reabsorption, mummification, autolysis, stillbirth, or the birth of weak underweight calves which may have symptomatic disease (ataxia, paralysis, exophthalmus (pop-eye)) or harbour chronic infections. Infections commonly involve neural tissues (brain, spinal cord, nerve roots, retina) where they cause inflammation (encephalomyelitis) and necrotic areas (focal malacia) with subsequent fibrosis, resulting in a range of neurological signs. Infections in cardiac tissues may cause focal or diffuse myocarditis, infections in skeletal muscles cause necrosis and myositis, and infections in the liver cause multifocal hepatocellular necrosis and periportal hepatitis. Chronically-infected calves are more likely to go on to produce infected calves or to experience abortion themselves. In aborted foetuses, parasites and inflammatory infiltrates are usually found in the brain, spinal cord and heart, or in the liver when abortion storms occur. Infections have been associated with both epidemic and endemic bovine abortions, conditions associated with horizontal and vertical transmission, respectively. Epidemic abortions follow postnatal exposure to infective oocysts, with parasites causing acute disease and typically being found in the hearts and livers of aborted foetuses with multifocal hepatocellular necrosis. Endemic abortions occur in chronically infected cattle when latent encysted parasites are reactivated during pregnancy and cross the placenta where they may be found in the brains of aborted foetuses. Infections by *N. hughesi* in horses are often subclinical but some may cause equine protozoal myeloencephalitis (EPM) resulting in generalized neurological symptoms such as ataxia, weakness, seizures, paralysis, vision problems, or behavioural changes.

Developmental cycle and mode of transmission: Experimental cross-transmission studies demonstrated that *N. caninum* had a heteroxenous (2-host) life-cycle involving cattle as intermediate hosts (IH) in which asexual proliferation occurred, and dogs as definitive hosts (DH) in which sexual reproduction took place. Case studies have now confirmed infections by tissue cysts in a range of herbivorous and omnivorous animals, as well as infections resulting in oocyst formation in a range of canids. Curiously, dogs may act as either DH producing oocysts or as IH with schizonts and cysts infecting tissues and sometimes causing systemic disease resulting in paralysis or abortion (similar to that occurring in ungulates). Transmission from IH to DH occurs horizontally (laterally, post-natally) via predator-prey interactions when infected flesh is eaten by canids, either by direct predation (eating infected IH), scavenging (eating infected remains, including carcases, aborted foetal tissues or placental tissues) or being fed flesh from farm ration animals (eating raw meat or carcase offcuts). It has been shown experimentally that both tachyzoites from schizonts and bradyzoites from tissue cysts may initiate infections in dogs. While transmission studies have not yet demonstrated all the stages involved in the enteric cycle in dogs (other than oocyst formation), they are thought to first involve an asexual schizont cycle then the formation of sexually-dimorphic gametocytes producing macrogametes (female) and flagellated microgametes (male) that undergo fertilization by fusion to form zygotes. Oocyst formation has been observed in intestinal epithelial cells resulting in the excretion of unsporulated oocysts in canid faeces. Sporulation occurs exogenously with the formation of sporocysts and sporozoites taking several days. The prepatent period (time interval from infection to first oocyst excretion) varies from 2-13 days, and the patent period (duration of oocyst excretion ranges from 8-27 days, but some reports suggest intermittent oocyst excretion may still occur after several months. The intensity of oocyst shedding depends on the age of the dogs (more in puppies) and the type of flesh they have consumed (more in dogs fed skeletal muscles from cattle, sheep or goats than those fed other tissues or infected mouse cadavers). Transmission from DH to IH occurs horizontally via faecal-oral interactions where oocysts contaminating food and water sources are consumed by omnivores/herbivores (mostly mammals but including some birds). Ingested oocysts excyst in the gut releasing sporozoites which invade host cells forming schizonts which produce numerous tachyzoites by asexual internal division (endodyogeny) over several days. Mature schizonts ultimately lyse their host cells releasing the contained tachyzoites to invade further cells. Several cycles of schizogony may occur facilitating the rapid increase in parasite numbers and their dissemination throughout host tissues. Eventually, the tachyzoites are transformed to begin bradyzoite production within intracellular membrane-bound cysts (transformation linked to strong gamma-interferon dominated cell-mediated host immune responses). Tissue cysts

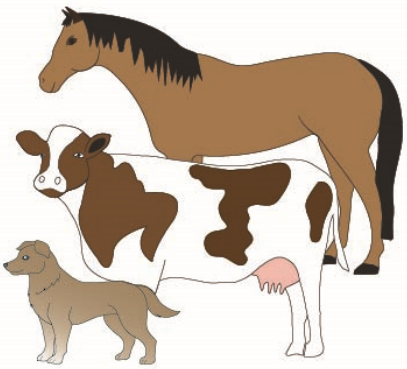
typically occur in neural or skeletal muscle cells where they may persist for several months or years. The life cycle is completed when mature cysts containing infective bradyzoites are eaten by canids. In addition to the heteroxenous (IH-DH) life-cycle, infections may also exhibit diheteroxenous (IH-IH) vertical transmission when tachyzoites cross the placenta in pregnant animals and invade foetal tissues. Transplacental infections may lead to the termination of pregnancy with abortion or stillbirth, but they may also lead to the birth of live offspring harbouring congenital infections. Such infections may occasionally cause systemic disease in young animals, but they often remain latent for long periods and become reactivated during gestation for several pregnancies (thought to be due to the transient immunosuppression developing in the host to avoid foetal rejection). Congenital infections therefore facilitate the transmission of infections between multiple generations of animals. Transplacental and congenital infections are considered to be the major routes of infection in domestic cattle (both beef and dairy herds). Epidemiological studies have indicated that infected cows have a 50-90% chance of congenital infection, with only some infections resulting in endemic abortion, although some herds occasionally experience abortion 'storms' around 4-7 months of gestation. In horses infected with *N. hughesi*, vertical transmission has been found between latently-infected mares and foals via endogenous transplacental transmission.

Differential diagnosis: *Neospora* infections may be indicated in animals on the basis of history and clinical signs (paralysis and/or abortion), but they must be confirmed (and differentiated from *Toxoplasma*) by the detection of parasites in clinical samples by microscopic examination or culture, the molecular detection of parasite DNA, or the demonstration of specific host antibodies against defined parasite antigens. Infections are usually provisionally diagnosed by the microscopic detection of schizonts and/or cysts in histological sections of tissues collected from deceased animals or freshly-aborted foetuses, notably neural tissues, striated muscles (heart, skeletal musculature) and sometimes the liver and lungs. The generic identity of the parasites is confirmed by staining with specific antiserum (which does not cross-react with *Toxoplasma* stages) tagged to fluorochrome or chromogenic labels. *Neospora* spp. in horses can sometimes be differentiated on the basis of cyst morphology, with *N. caninum* cysts usually being larger than those of *N. hughesi* and being bound by thicker cyst walls. Infections in canid definitive hosts are diagnosed by the coprological examination of faecal samples for the presence of characteristic oocysts (isosporid-like with 1:2:4 configuration) following concentration by sedimentation-floatation techniques. Regrettably, differential diagnosis is confounded by other parasite genera having similar oocysts. *Neospora* oocysts (from canids) are identical to those of *Toxoplasma gondii* (found in felids) and *Hammondia* spp. (found in canids and felids). Several bioassays have been developed to study parasites cultured *in vitro* in cell cultures (e.g. Vero cells in RPMI-1640 media, bovine aorta endothelial cells, murine epidermal keratinocytes, and organotypic slice explants of rat brain cortex) or *in vivo* in laboratory mice (Swiss white mice, BALB/c, CBA/ca, ICR, interferon-gamma gene knockout strains) or gerbils. A range of serological tests (agglutination tests, indirect fluorescent-antibody tests (IFAT), enzyme immunoassays) have been developed to detect specific host antibodies (in serum or cerebrospinal fluid) against parasite antigens using either whole zoites, sonicate extracts or defined recombinant antigens (notably P37 and P29/30 immunodominant antigens). The different tests varied in their sensitivity and specificity depending on the host species and samples tested, but the IFAT was found to provide the most reliable results (and has been adopted by some authorities as the diagnostic standard). More recently, molecular biological techniques have been used to detect and characterize parasites following the polymerase chain reaction (PCR) amplification of nuclear gene sequences (18S ribosomal DNA, internal transcribed spacer region 1, alpha-tubulin, beta-tubulin, heat shock protein 70, repetitive DNA (Nc5 repeat sequence), by random amplified polymorphic DNA (RAPD) analyses, or by mini- and micro-satellite analyses.

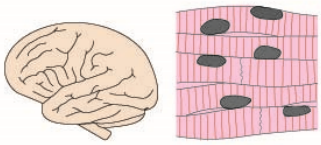
Treatment and control: Once clinical disease has become apparent, there is currently no effective treatment for neosporosis. The prognosis in dogs with hindlimb paralysis is poor, although some early cases have responded to treatments with various combinations of lincosamide antibiotics (clindamycin) and antiprotozoals including diaminopyrimidines (trimethoprim), sulfonamides (sulfadiazine) and diaminopyrimidines (pyrimethamine). Cattle have been treated with clindamycin and triazine-based coccidiostats (toltrazuril, ponazuril) with variable effects but treatments were costly and there were mandated withholding periods for meat and milk. Various preventive measures have been implemented to interrupt transmission cycles on farms through improved hygiene and sanitation as well as to manage herd health through husbandry practices. To prevent oocyst production, it is important to deny dogs access to raw meat, carcases, aborted foetuses and placentas by restricting their roaming and scavenging behaviours and maintaining them on diets involving cooked/processed meats. Programs could also be instituted to control wild or feral animals (dogs, dingoes and foxes) using barriers, baits or by hunting. Domestic dogs should be prevented from defaecating on or near fodder storage facilities, animal enclosures and water sources and efforts should be made to keep livestock feeding and drinking areas clean by regular washing/flushing. Health surveillance programs should be established to monitor for clinical cases and all new or replacement livestock should be screened for infections. Serological testing of whole herds may also be used to select sero-negative animals for breeding and possibly to cull sero-positive animals (impractical in herds with high levels of infection – most often only aborting animals are culled). Several vaccines have been developed for use in cattle, but they are relatively expensive and while they reduce the rate of abortion, they do not prevent vertical transmission. The treatment of *N. hughesi* in horses is difficult as diagnosis is often made post-mortem, and there may be latent infections in other horses without clinical symptoms. Some successes have been reported treating horses with triazine-based coccidiostats (toltrazuril, ponazuril). Serological screening horse populations may identify infected individuals which could be selectively culled but often little is done due to the rarity of symptomatic disease.

Neospora

heteroxenous (2-host) cycle
predator-prey transmission
(predator ingests tissue cysts in prey)
(prey ingests sporocysts shed by predator)
plus vertical transmission in prey
(transplacental/transmammary)



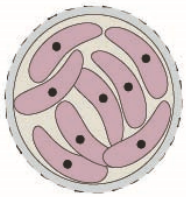
Intermediate Hosts (IH)
(mammals, esp. ungulates)

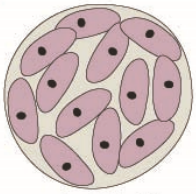


brain, muscles
(space-occupying lesions,
ascending paralysis,
abortion, neonatal
mortalities)

asexual merogony
(disseminated)

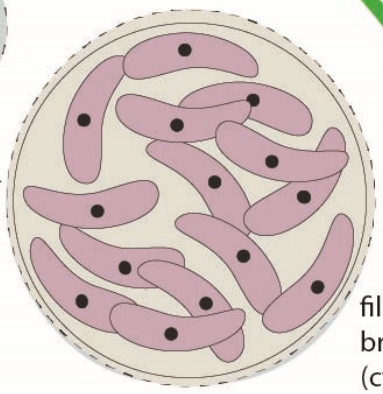
tissue cyst formation
in nerve/muscle cells





meronts (10-20 µm)
(filled with merozoites
(called tachyzoites))

thin PAS+
cyst wall

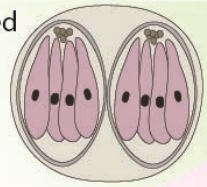


filled with
bradyzoites
(cystozoites)

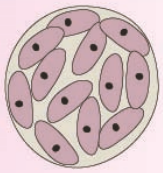
tissue cysts
(10-100 µm)

excystation

oocysts
ingested



oocyst
(9-13 µm)
sporocysts
(7-9 µm,)



tissue cysts
ingested

meronts (8-12 µm)

exogenous
sporulation

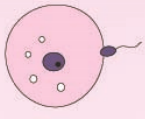
oocysts
excreted

sporoblast

male
gamonts (~ 10 µm)

female

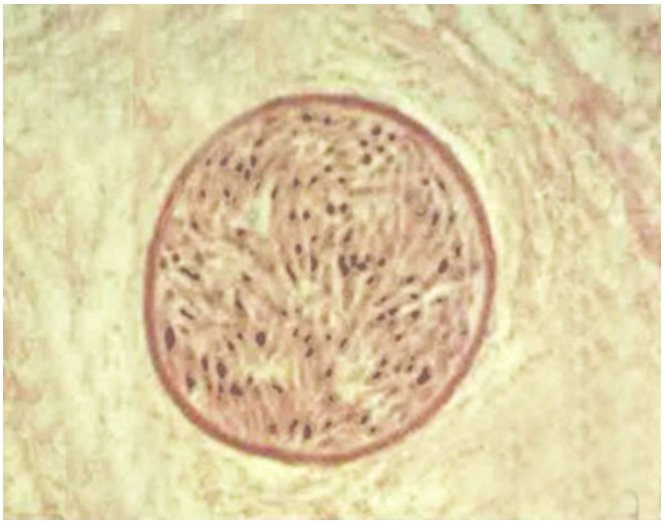
sexual
gamogony



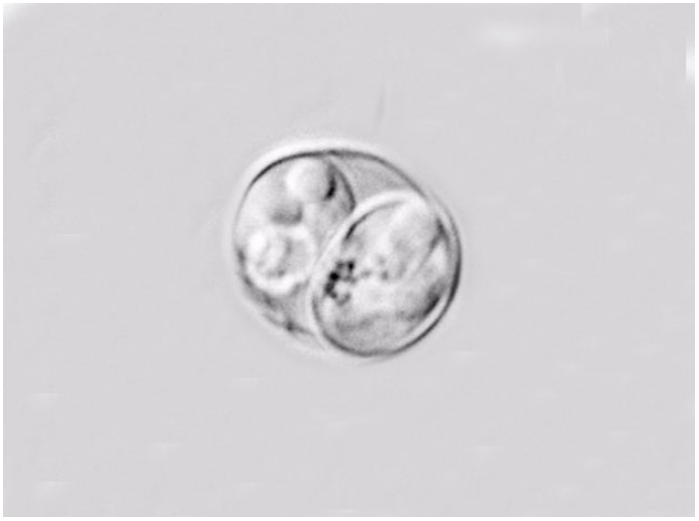
fertilization

Definitive Hosts
(canids)
(intestines)

oocyst:sporocyst:sporozoite
configuration = 1:2:4



Neospora cyst in cow brain



Neospora oocyst from dog faeces