# Neosporosis, Toxoplasmosis, and Sarcocystosis in Ruminants: An Update

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## KEYWORDS

- *Neospora caninum* • *T gondii* • *Sarcocystis* spp. • Cattle • Sheep • Goats
- Water buffalo • White-tailed deer

## KEY POINTS

- Neosporosis, toxoplasmosis, sarcocystosis are parasitic diseases that are transmitted to ruminants by oocysts shed in the feces of a carnivore or omnivore definitive hosts.
- Neosporosis causes of abortion in cattle, dogs are definitive hosts; it is transmitted transplacentally, and this is the major way it is transmitted in cattle.
- Toxoplasmosis is a cause of abortion in sheep and goats; cats are the definitive hosts.
- Sarcocystosis can cause abortion and carcass condemnation in ruminants. Dogs, cats, and wild predators are definitive hosts.
- No effective preventative chemotherapeutics or vaccines are available for these parasites in ruminants in North America.

## NEOSPOROSIS
### Etiology

Neosporosis is a disease caused by the *Toxoplasmosis gondii*-like parasite, *Neospora caninum*. Until 1988, *N caninum* was confused with the structurally similar coccidian *T gondii*.[1] The disease was recognized and the features of the clinical disease it causes in congenitally infected dogs was reported in dogs from Norway in 1984.[2] This report prompted a retrospective study that provided a scientific description of the parasite and the name *N caninum*.[3] The parasite was isolated in cell cultures from congenitally infected Labrador retriever pups.[4] This process provided a source of antigen for immunologic[4] and immunohistochemical[5] studies. Researchers used these tools and within a few years it became apparent that neosporosis was an important cause

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of abortion and neonatal mortality in cattle-rearing regions worldwide.[1] In 1998, domestic dogs were found to be the definitive host and also excrete oocysts.[6,7] Coyotes and wolves[8,9] are also definitive hosts and maintain the parasite in a sylvatic cycle with white-tailed deer (*Odocoileus virginianus*) where they are present.[10] Viable *N caninum* has been isolated from dogs, cattle, white-tailed deer, water buffaloes, and sheep.[1] *N caninum* is not known to naturally infect primates or humans, but experimental infections in rhesus macaque tachyzoite crossed the placenta and infected the fetus.[1]

The life cycle is typified by 3 infectious stages: tachyzoites, tissue cysts, and oocysts (Fig. 1). Tachyzoites disseminate the infection extracellularly by moving between host cells or via the blood. Tissue cysts are latent stages found in the intermediate hosts and both occur intracellularly in vacuoles derived from the host cell plasma membrane.[1,3,4] Tachyzoites are approximately 6 × 2 μm. They are rapidly dividing stages that cause tissue damage, disseminate the infection in the intermediate host and are transplacentally transmitted to the fetus. Tachyzoites divide asexually into 2 organisms by a type of longitudinal binary fission called endodyogeny. The tachyzoites eventually produce bradyzoites by endodyogeny after receiving a cue from the host to undergo stage conversion to produce the dormant thick-walled tissue cyst stage.
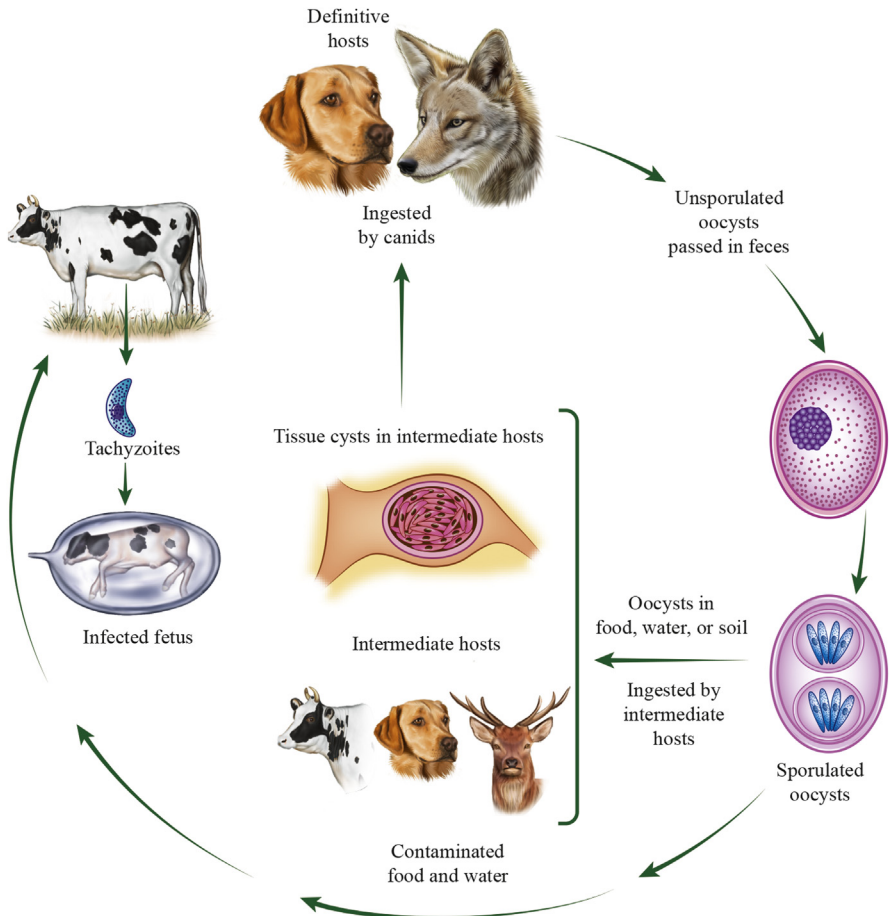


**Fig. 1.** Life cycle of *Neospora caninum*. (*Courtesy of* J. P. Dubey, MVSc, PhD, Beltsville, MD.)

Tissue cysts are seen detected in the central nervous system.[11] These tissue cysts are round or oval in shape, up to 107 μm long, and contain up to 100 slow-growing bradyzoites. The tissue cyst wall is up to 4 μm thick and the enclosed bradyzoites are 7 to 8 × 2 μm. Bradyzoites are believed to produce asexual and sexual stages in the intestines of canines that results in oocysts in their feces.

*N caninum* oocysts are excreted unsporulated in the feces and measure 10 to 12 μm in diameter and sporulation occurs outside the host.[6] Presently, little is known regarding the frequency of shedding of *N caninum* oocysts, the survival of the oocysts in the environment, and how many different canines can serve as definitive hosts.[1] Oocysts are most likely susceptible to environmental conditions that inhibit oocysts of *T gondii*. The parasite can be transmitted transplacentally in several hosts and the vertical route is the major mode of its transmission in domestic dairy antibody cattle.[11] There is no adult cow to adult cow transmission of *N caninum*. Although most *N caninum* infections in cattle are transmitted transplacentally, reports of postnatal rates have been variable depending on the region of the country, the type of test used, and the antibody cut-off values used.[1,11] Although *N caninum* has been found in bovine semen,[12] it is unlikely that *N caninum* is transmitted in semen or by embryo transfer from the donor cows. Embryo transfer has been recommended as a method of control to prevent vertical transmission.[13] However, it is prudent to test all recipients, and embryos should not be transferred to seropositive cows. Lactogenic transmission of *N caninum* is considered unlikely.[14–16] Canids can acquire infection by ingestion of infected tissues.

### Neosporosis in Cattle

#### Clinical signs

*N caninum* is responsible for inducing abortion both dairy and beef cattle.[1,17–20] Cows of any age may abort from 3-month gestation to near full term. Most neosporosis-induced abortions occur at 5 to 6 months of gestation. Fetuses may die in utero, be resorbed, mummified, autolyzed, stillborn, born alive with clinical signs, or born clinically normal but chronically infected. Neosporosis-induced abortions occur year round. Cows with *N caninum* antibodies (seropositive) are more likely to abort than seronegative cows and this applies to both dairy and beef cattle. However, up to 95% of calves born congenitally infected from seropositive dams remain clinically normal. The age of dam, lactation number, and history of abortion generally do not affect rate of congenital infection, but there are reports indicating that in persistently infected cattle vertical transmission is more efficient in younger than older cows. If replacement heifers are infected, they may either abort or transplacentally infect their offspring.

Clinical signs have only been reported in cattle younger than 2 month of age.[1,11] *N caninum*-infected calves may have neurologic signs, be underweight, unable to rise, or be born without clinical signs of disease. Hind limbs or forelimbs or both may be flexed or hyperextended. Neurologic examination may reveal ataxia, decreased patellar reflexes, and loss of conscious proprioception. Calves may have exophthalmia or asymmetrical appearance in the eyes. *N caninum* occasionally causes birth defects including hydrocephalus and narrowing of the spinal cord.[1]

Abortions may be epidemic or endemic.[17–20] Up to 33% of dairy cow abortions owing to *N caninum* occur within a few months of pregnancy. Abortions are considered epidemic if more than 10% of cows at risk have aborted within 6 to 8 weeks. A small proportion (<5%) of cows are reported to have repeated abortions owing to neosporosis.[21] Cows with *N caninum* antibodies (seropositive) are more likely to abort than seronegative cows. There is an increase in antibody titers 4 to 5 months before

parturition. These observations strongly suggest reactivation of latent infection; however, little is known regarding the mechanism of reactivation. It is likely that there is parasitemia during pregnancy leading to fetal infection. However, N caninum has never been identified in histologic sections of adult cows and viable N caninum has been isolated from the brains of only 2 cows.[22,23] Although it is reasonable to speculate that pregnancy-induced immunosuppression or hormonal imbalance may reactivate latent tissue cysts of N caninum, such a mechanism has not been demonstrated experimentally or in natural infections. N caninum DNA has been found in blood of naturally infected cattle indicating parasitemia.[24] N caninum is one of the most efficiently transplacentally transmitted organisms in cattle. In some herds, up to 90% of cattle are infected, and most calves born congenitally infected with N caninum remain healthy.

### Prevalence

N caninum infections have been reported from most parts of the world. Serologic prevalence in cattle varies, depending on the country, region, type of serologic test used, and antibody titer cut-off level used to determine exposure. In some dairies up to 87% of cows are seropositive[1] and studies involving a large number of fetuses in many countries indicate that 12% to 42% of aborted fetuses from dairy cattle are infected with N caninum.[1] Less is known of the causes of abortion in beef cattle than in dairy cattle owing to the difficulty of finding small aborted fetuses expelled in the field during the first trimester. There is no evidence of N caninum-associated morbidity in beef cattle more than 2 months of age.[1]

### Diagnosis

Examination of the serum from an aborting cow is only indicative of exposure to N caninum and histologic examination of the fetus is necessary for a definitive diagnosis of abortion owing to neosporosis.[25] The brain, heart, liver, placenta, and body fluids or blood serum are the best specimens for diagnosis and diagnostic rates are higher if multiple tissues are examined.[25] Although lesions of neosporosis are found in several organs, fetal brain is the most consistently affected organ.[25] Because most aborted fetuses are likely to be autolyzed, even semiliquid brain tissue should be fixed in 10% buffered neutral formalin for histologic examination of hematoxylin and eosin–stained sections. Immunohistochemistry is necessary because there are generally only a few N caninum present in autolyzed tissues and these are often not visible in hematoxylin and eosin–stained sections. The most characteristic lesion of neosporosis is focal encephalitis characterized by necrosis and nonsuppurative inflammation.[25] Hepatitis is more common in epizootic than sporadic abortions.[26] Lesions are also present in the placenta, but protozoa are difficult to find.

The efficiency of the diagnosis by polymerase chain reaction (PCR) depends on the laboratory, stage of the autolysis of the fetus, and sampling procedures.[25,27,28] Fresh or frozen tissues are superior to formalin-fixed tissues. N caninum DNA can be detected by PCR in formalin-fixed, paraffin-embedded bovine tissue, but it is less sensitive than PCR on fresh/frozen tissue.

Serologic tests can be used to detect N caninum antibodies including various enzyme-linked immunosorbent assays (ELISAs), the indirect fluorescent antibody test, and the Neospora agglutination test.[1,29,30] There are several modifications of the ELISA test to detect antibodies to N caninum in sera or milk using whole parasite, whole parasite lysate, purified proteins, recombinant proteins, tachyzoite proteins absorbed on immunostimulating complex adjuvant particles and some of these teats were compared recently in a multicentered study in various laboratories in Europe.[31]

Avidity ELISAs designed to distinguish acute and chronic infections in cattle seem to be promising to distinguish endemic and epidemic abortion.[32] In the avidity ELISA sera are treated with urea to release low-avidity (low-affinity) antibodies and differences in values obtained before and after treatment with urea are used to evaluate recency of infection. In recently acquired infection, avidity values are low.[32] Another modification of ELISA is antigen capture. This test detects (captures) a 65-kD antigen in sera of infected cattle using a specific monoclonal antibody and this test is commercially available.[33] Immunoblots are useful in detecting *N caninum*-specific antibodies.[25]

Finding *N caninum* antibody in serum from the fetus can establish *N caninum* infection, but a negative result is not informative because antibody synthesis in the fetus depends on the stage of gestation, level of exposure, and the time between infection and abortion. Although blood, serum, or other body fluids from the fetus may be used for serologic diagnosis, peritoneal fluid is better than other body fluids. In calves, pre-suckling serum can be submitted for diagnosis of congenital infection.

The definitive antibody level that should be considered diagnostic for neosporosis has not been established for bovines because of the uncertainty of serologic diagnosis in chronically infected animals and the availability of sera from noninfected cattle. In serologic assays, titer and absorbance values depend on antigen composition, secondary antibodies, and other reagents.[25] Further, antibody titer cut-off levels can be arbitrarily selected to provide sensitivity and specificity requested for a particular application. The age and class of an animal may also affect selection of an antibody titer cut-off level. Although *N caninum* is closely related to *T gondii*, cut-off titers in general are higher in cattle that have aborted owing to neosporosis than those with normal pregnancy; however, titers in individual cows cannot determine the etiology of abortions.

### Control

*N caninum* is efficiently transmitted vertically in cattle, perhaps for several generations. Culling is 1 way at present to prevent this transmission from cow to heifer.[34] However, culling is impractical if the prevalence of *N caninum* in a herd is very high. Before making the decision to cull, it is advisable to estimate the prevalence of *N caninum* in the herd. Bulk milk testing can provide preliminary data about the prevalence of *N caninum* infection. If a bulk milk test is positive, antibody prevalence in dam-heifer samples and cattle of different ages can provide insight to the transmission of *N caninum* in a given herd. In herds with a high transplacental transmission, the prevalence of *N caninum* in cattle of different ages is about the same and there is high correlation between infection in dams and daughters. To decrease vertical transmission of *N caninum*, culling of seropositive dams and/or heifer calves from seropositive cows, and embryo transfer from seropositive cows to seronegative cows are some management strategies that can be adapted. There are no drugs that kill *N caninum* bradyzoites within tissue cysts.

To prevent horizontal (from outside sources) transmission, it is important to prevent exposure of the cows to feed and water contaminated with oocysts.[35,36] Domestic dogs and other canids should not be allowed to defecate in cattle feed, barns, or pasture, although this is not easy to achieve. How dogs become infected with *N caninum* is not well-understood. Consumption of aborted bovine fetuses does not seem to be an important source of *N caninum* infection in dogs. The consumption of placental membranes may be a source of *N caninum* infection in dogs because the parasite has been found in naturally infected placentas and dogs fed placentas shed *N caninum* oocysts.[16] Little is known at present regarding the frequency of

shedding of *N caninum* oocysts by canids in nature, the resistance of the oocysts, or whether dogs shed oocysts more than once. Domestic and wild canines should not be allowed to eat aborted fetuses, fetal membranes, or dead calves. Other factors such as farm location can be a risk factor.[36] There is evidence that cattle can develop protective immunity to subsequent neosporosis abortion.[37,38] This protective immunity seems to be more effective in cows that are subsequently infected with an exogenous source (oocysts) than in cows in which there is a recrudescence of a persistent infection.[38] Inducing protective immunity through vaccination against abortion in cows that already harbor a latent infection is problematic.

The only commercial *N caninum* vaccine (Neo Guard) has been removed from the market because of lack of convincing data about the efficiency of the vaccine to prevent *N caninum* associated abortions in cattle.[39,40]

## NEOSPOROSIS IN OTHER ANIMALS

Neosporosis is a primary disease of dogs. In addition to dogs and cattle, sporadic cases of clinical neosporosis have been reported in other animals including adult horses, in a 16 day-old rhinoceros (*Ceratotherium simum*), in a juvenile raccoon (*Procyon lotor*), in a 2-month-old black-tailed deer (*Odocoileus hemionus columbianus*), in neonatal alpacas (*Vicugna pacos*) and llamas (*Lama glama*), goats, sheep, Eld's deer (*Cervus eldi siamensis*), Fallow deer (*Dama dama*), and an antelope (*Tragelaphus imberbis*).[1] A new species, *Neospora hughesi*, has been described in horses.[41] It is molecularly different from *N caninum*[42,43] and tissue cysts of *N hughesi* were not infectious for dogs.[44] It is presently not known if *N caninum* infects horses or *N hughesi* infects ruminants or other animals.

## TOXOPLASMOSIS
### Etiology

Toxoplasmosis is caused by the infection with the protozoan *T gondii*.[45] It is among the most common of parasites of animals and *T gondii* is the only known species. Felids are the definitive hosts, and warm-blooded animals are intermediate hosts.[45] There are 3 infectious stages of *T gondii* for all hosts: tachyzoites (individually and in groups), bradyzoites (in tissue cysts), and sporozoites (in sporocysts within sporulated oocysts) (Fig. 2).[46]

The tachyzoite and bradyzoite stages of *T gondii* are morphologically and biologically nearly identical to those of *N caninum*.[1,45,46] The tachyzoites are metabolically active and are susceptible to agents used to treat coccidia infections. The slow-growing bradyzoites in tissue cysts are not as metabolically active and are not affected by drugs used to treat coccidia. Tissue cysts grow and remain intracellular. They vary in size from 5 to 70 μm and contain a few to several hundred bradyzoites.[46,47] Although tissue cysts may develop in visceral organs, including lungs, liver, and kidneys, they are more prevalent in muscular and neural tissues, including the brain, eye, skeletal, and cardiac muscle. Intact tissue cysts of *T gondii* are probably harmless and can persist for the life of the host.[45]

The tissue cyst wall is elastic, thin (<0.5 μm), and may enclose hundreds of crescent-shaped slender bradyzoites each measuring $7.0 \times 1.5$ μm. Bradyzoites differ only slightly structurally from tachyzoites in having a nucleus situated toward the posterior end whereas the nucleus in tachyzoites is more central. Bradyzoites are more slender than are tachyzoites and less susceptible to destruction by acid conditions and proteolytic enzymes in the stomach than tachyzoites. Tissue cysts are believed to periodically release bradyzoites and quickly destroyed by the hosts immune system contributing to life-long exposure and ongoing immunity to the parasite.
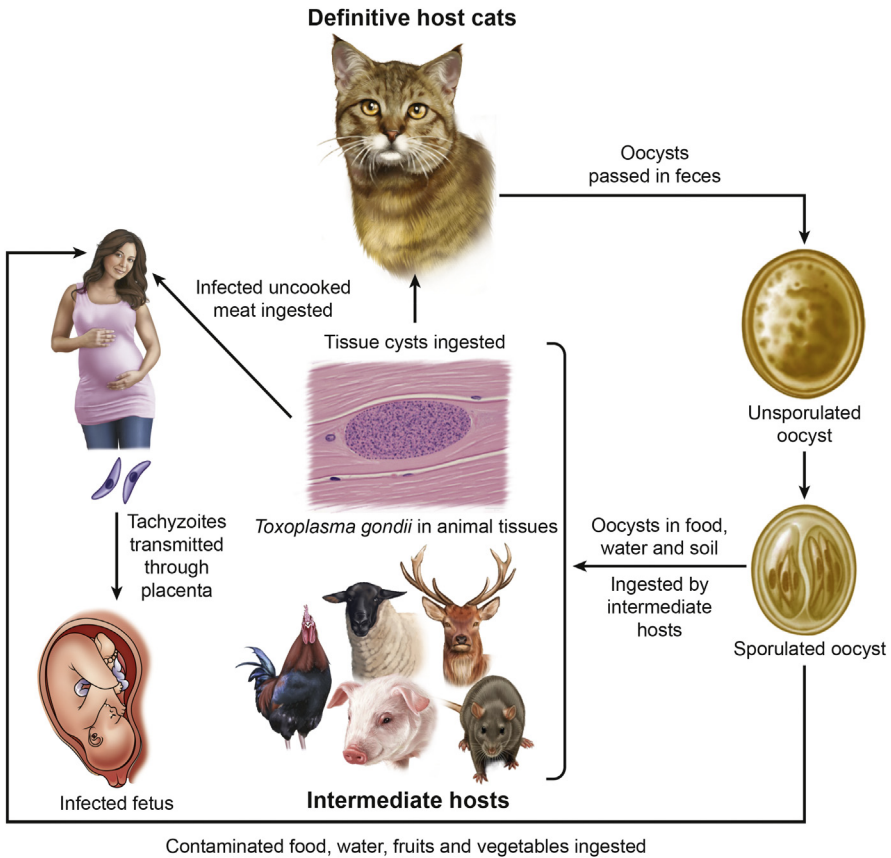
**Fig. 2.** Life cycle of *Toxoplasma gondii*. (*Courtesy of* J. P. Dubey, MVSc, PhD, Beltsville, MD.)

Upon ingestion by cats, the wall of the tissue cyst is digested and bradyzoites are released. Some penetrate the lamina propria of the intestine and multiple as tachyzoites.[47] Within a few hours, *T gondii* may disseminate to extraintestinal tissues. Other bradyzoites penetrate epithelial cells of the cat small intestine and initiate 5 cycles (types A to E schizonts) of schizonts[47,48] and then become sexual stages. Some become male stages (microgamonts) and produce microgametes (sperm) but most become female stages that will be fertilized by the microgametes and will form a zygote. The zygote produces an oocyst wall around the sporont. When oocysts are mature, they are discharged into the intestinal lumen by the rupture of intestinal epithelial cells.

Oocysts of *T gondii* are formed only in cats, including both domestic and wild felids. Domestic cats shed oocysts after ingesting tachyzoites,[49] bradyzoites,[47] or sporozoites.[50] However, less than 50% of cats shed oocysts after ingesting tachyzoites or oocysts, whereas nearly all shed oocysts after ingesting tissue cysts.[47,49,50]

Oocysts in freshly passed feces are unsporulated (noninfective) and subspherical or spherical in shape and 10 to 12 × 10 to 12 μm in diameter. Sporulation occurs outside the cat and within 1 to 5 days, depending on humidity, oxygen, and temperature. Sporulated oocysts contain 2 ellipsoidal sporocysts. Each sporocyst contains 4 sporozoites. The sporozoites are 6 to 8 × 2 μm in size.

Hosts, including felids can acquire *T gondii* by ingesting either tissue cysts in tissues of infected animals or sporulated oocysts in food or drink, or by transplacental transmission of tachyzoites from mother to fetus. After ingestion, bradyzoites released from tissue cysts or sporozoites from oocysts penetrate intestinal tissues, transform to tachyzoites, multiply locally as tachyzoites, and are disseminated in the body via blood or lymph as tachyzoites to leukocytes that contain viable tachyzoites. After a few multiplication cycles, tachyzoites give rise to bradyzoites in a variety of tissues and undergo stage transformation to produce tissue cysts. *T gondii* infection during pregnancy can lead to infection of the fetus. Congenital toxoplasmosis in sheep and goats can kill the fetus. Oocysts are more pathogenic than tissue cysts for hosts during a primary infection and edema, necrosis of the lamina propria, and sloughing of the intestinal mucosa can produce severe enteritis.

### Host–Parasite Relationship

*T gondii* can multiply in most nucleated cell types in the body. How *T gondii* stages are destroyed by immune cells is not completely known. All extracellular forms of the parasite are directly affected by antibodies, but intracellular forms are not. Cellular factors, including lymphocytes and lymphokines, are thought to be more important than humoral factors (antibodies) in the immune-mediated destruction of *T gondii* stages in hosts.

Acquired immunity does not eliminate an established infection, but tachyzoites stages convert to bradyzoites in response to a host stimulus and develop into tissue cysts. During early stage conversion and production of tissue cysts, tachyzoites and bradyzoites can be seen in the same developing tissue cyst. As the tissue cyst matures, the numbers of tachyzoites-like stages become less and eventually only bradyzoites are present in the tissue cyst. *T gondii* tissue cysts persist for several years after acute infection. The fate of tissue cysts residing in an immunocompetent host is not fully known. Some tissue cysts may rupture during the life of the host and the released bradyzoites are destroyed by the host's immune responses locally. However, in immunosuppressed individuals, infection can be reactivated and bradyzoites stages convert to tachyzoites, which leads to dissemination of infection in the host.

The pathogenicity of *T gondii* is determined by many factors, including the innate susceptibility of the host species, virulence of the parasitic (its genotype), and the stage that is acquired by the host.[45] Oocyst-induced infections are the most severe clinically in intermediate hosts, and this is not dose dependent.[45] *T gondii* genotypes differ remarkably in their virulence to outbred mice. However, the virulence of *T gondii* in mice does not always equate to virulence in domestic animals.

*T gondii* has also adapted to an oocyst–oral cycle in herbivores (intermediate hosts) because these animals do not consume tissue cysts. The tissue cyst–oral cycle in carnivores and omnivores is efficient and a means to be maintained the life cycle when cat populations are low. Epidemiologic evidence indicates that cats are essential in perpetuation of the life cycle as *T gondii* infection is rare or absent in areas devoid of cats.[51–53] *T gondii* oocysts are less infective and less pathogenic for cats than for mice.[45]

### Epidemiology

Domestic cats are the major source of contamination of the environment with oocysts, as they are more common than wild fields (bobcats) and produce large numbers of *T gondii* oocysts.[45] Sporulated oocysts survive for long periods under moderate environmental conditions and can be spread by erosion of topsoil, and mechanically by flies, cockroaches, dung beetles, and earthworms.

Although only a few cats may be shedding *T gondii* oocysts at any given time the millions produced by each infected cat and their resistance to destruction assure widespread environmental contamination.[54] Seroprevalence in cats is largely determined by the prevalence of infection in the local avian and rodent populations, which serve as prey. For epidemiologic surveys, seroprevalence data for cats are more useful than results of fecal examination because cats with antibodies have likely shed oocysts and are an indicator of environmental contamination.[45]

### Clinical Toxoplasmosis

*T gondii* is capable of causing severe disease in small ruminants and is responsible for great losses to the livestock industry.[45] In sheep and goats, primary maternal infections may cause embryonic death and resorption, fetal death and mummification, abortion, stillbirth, and neonatal death. The disease is more severe in goats than in sheep. Cattle and water buffaloes are more resistant to acute clinical toxoplasmosis than are other species of livestock and there are no confirmed reports of clinical toxoplasmosis in these animals.

*T gondii* infection is widespread in humans and prevalence varies with geography and increases as a population ages. In the United States and the UK it is estimated that 16% to 40% of people become infected, whereas in Central and South America and continental Europe infection estimates reach 50% to 80%.[45,55] Infections in healthy adults are usually asymptomatic; however, severe disease can occur in immunocompromised individuals and newborns. Congenital infection may occur following maternal infection during pregnancy.[56,57] The severity of the disease depends on the immune status of the mother, parasite genotype,[58] and stage of pregnancy at the time of infection.[56–58] A wide spectrum of clinical disease occurs in congenitally infected children.

### Diagnosis

Diagnosis can be made by biological, serologic, molecular, or histologic methods or by a combination of these methods.[45] Clinical signs are nonspecific and insufficiently characteristic for a definite diagnosis because toxoplasmosis mimics several other infectious diseases.

Numerous serologic procedures are available for use in diagnostic laboratories for the detection of humoral antibodies, including indirect hemagglutination assays, indirect fluorescent antibody assays, direct agglutination tests, latex agglutination tests, ELISA, and the immunosorbent agglutination assay test.[45,57] The indirect fluorescent antibody assays, immunosorbent agglutination assay test, and ELISA have been modified to detect IgM antibodies, which appear sooner after infection than IgG and disappear faster than IgG after recovery. The finding of antibodies to *T gondii* in 1 serum sample merely establishes that the host has been infected at some time in the past, so it is best to collect 2 samples from the same individual, the second 2 to 4 weeks after the first.[45] A 4- to 16-fold increase in antibody titer in the second sample indicates an acute infection. A high antibody titer sometimes persists for months after infection. Tissues samples submitted to diagnostic laboratories can be examined by immunohistochemical staining for *T gondii* or for PCR detection of parasite DNA.

### Chemotherapy

Sulfadiazine and pyrimethamine are widely used for therapy of human and animal toxoplasmosis.[45,57] These drugs act synergistically by blocking the metabolic pathway involving *p*-aminobenzoic acid and the folic–folinic acid cycle, respectively. The drugs are usually well-tolerated; sometimes thrombocytopenia or leukopenia

may develop, but these effects can be overcome by administering folinic acid and yeast without interfering with treatment, because the vertebrate host can transport presynthesized folinic acid into its cells, whereas *T gondii* cannot. Although these drugs have a beneficial action when given in the acute stage of the disease, when there is active multiplication of the parasite, they will not usually eradicate infection. Spiramycin, clindamycin, atovaquone, azithromycin, roxithromycin, clarithromycin, dapsone, and ponazuril and several other less commonly used drugs are available for treatment of toxoplasmosis, but none are approved for this purpose and restrictions on their use in food animals may limit their usage. Clindamycin is absorbed quickly and diffuses well into the central nervous system and therefore, has been used as alternative to sulfadiazine.[45,57]

### Prevention and Control

It is difficult to prevent cats from being on farms that have grazing stock. In the farm environment, young cats that have recently been weaned are more likely to be passing *T gondii* oocysts than are older cats that have been on the farm for several months to years. The producer should take measures to control rodents and wild birds to help decrease the source of potentially infected prey. Cats should be prevented from entering feed storage areas.

### Vaccination

There is no commercial vaccine to prevent *T gondii* infection in ruminants in North America. One live vaccine that contains a genotype (S48) of *T gondii* that does not persist in the tissues of sheep is available in Europe and New Zealand, where it is used to decrease fetal losses attributable to toxoplasmosis.[59] Ewes vaccinated with the S48 strain vaccine retain immunity for at least 18 months.[59] The S48b is not for use in pregnant ewes.

## SARCOCYSTOSIS
### Etiology

Unlike *N caninum* and *T gondii,* the genus *Sarcocystis* is much more diverse in the types of animals that can serve as definitive hosts (and excrete oocysts) and the types of animals that can serve as intermediate hosts (and contain the sarcocyst) and be eaten by the definitive host.[60] The genus *Sarcocystis* contains more than 100 named species that cycle between mammals, marsupials, birds, and reptiles as either the definitive host or the intermediate host. *Sarcocystis* has an obligatory prey–predator (2-host) life cycle (Fig. 3). Oocysts are passed fully sporulated by the definitive host and can be confused with the sporulated oocysts of *N caninum*, *T gondii*, and *Cystoisospora* spp. because they have 2 ellipsoidal sporocysts that enclose 4 sporozoites. Asexual stages develop only in the intermediate host, which in nature is often an herbivore (prey animal), and sexual stages develop only in the definitive host, which is a carnivore or omnivore. There are different intermediate and definitive hosts for each species of *Sarcocystis*; for example, there are 5 named species of *Sarcocystis* in cattle: *Sarcocystis cruzi, Sarcocystis heydorni, Sarcocystis hirsuta, S hominis, and Sarcocystis rommeli*, the definitive hosts for these species being canines (*S cruzi*), felines (*S hirsuta, S rommeli*), and primates (*S heydorni, S hominis*), respectively. Species of *Sarcocystis* parasites are generally more specific for their intermediate hosts than for their definitive hosts; for *S cruzi*, for example, ox and bison are the only intermediate hosts whereas dogs, wolves, coyotes, raccoons, jackals, and foxes can act as definitive hosts. In the following description of the life cycle and structure, *S cruzi* will serve as the example because its complete life cycle is known from experimental infections of intermediate and definitive hosts.
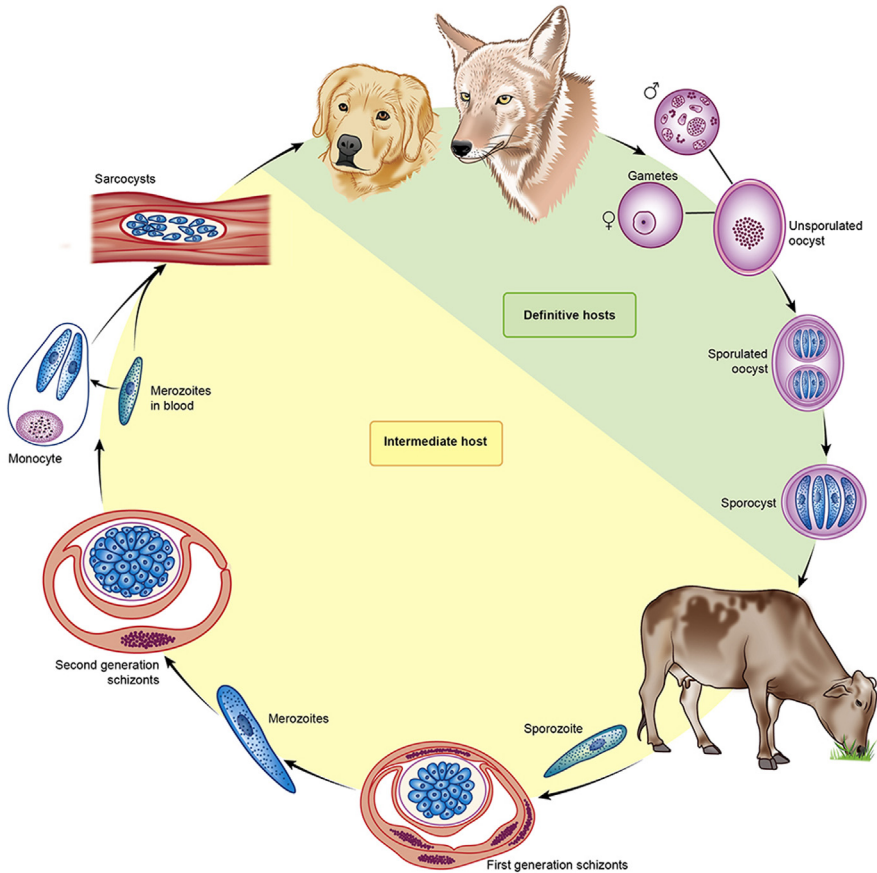
**Fig. 3.** Life cycle of *Sarcocystis cruzi*. (*Courtesy of* J. P. Dubey, MVSc, PhD, Beltsville, MD.)

The intermediate host becomes infected by ingesting sporocysts in food or water. Sporozoites excyst from sporocysts in the small intestine and the first generation of schizonts are formed in endothelial cells of arteries 7 to 15 days after inoculation while the second generation of schizonts occur 19 to 46 days after inoculation in capillaries throughout the body. Some merozoites are can also be found in mononuclear blood cells 24 to 46 days after inoculation. Both generations of schizonts develop asexually by a type of schizogony (asexual division) called endopolygeny, wherein the nucleus becomes lobulated and divides into several nuclei. Merozoites form at the periphery of the schizont. Both generation schizonts are located within the host cytoplasm and are not surrounded by a parasitophorous vacuole. Merozoites liberated from the last generation of schizonts initiate sarcocyst formation after penetration of appropriate host cells (striated and cardiac muscle, and occasionally the central nervous system). The intracellular merozoites are surrounded by a parasitophorous vacuole, unlike developing schizonts. The merozoite becomes a round to ovoid metrocyte and undergoes repeated division producing many metrocytes that eventually produce bradyzoites. As the sarcocyst matures, the numbers of bradyzoites increase and the numbers of metrocytes decrease. Eventually the sarcocyst is filled with bradyzoites. Sarcocysts generally become infectious about 75 days after infection, but there is

considerable variation among species of *Sarcocystis*. Immature sarcocysts containing only metrocytes are not infectious for the definitive host.

The definitive host becomes infected by ingesting tissues containing mature sarcocysts. Bradyzoites liberated from the sarcocyst by digestion in the stomach and intestine penetrate the mucosa of the small intestine and transform into male (micro) and female (macro) stages and after fertilization of a macrogamete by a microgamete a wall develops around the zygote and an oocyst is formed. The entire process of sexual development and fertilization can be completed within 24 hours.

Oocysts of *Sarcocystis* species sporulate in the lamina propria. Sporulated oocysts are thin walled (<1 μm). The thin oocyst wall often ruptures, releasing the sporocysts into the intestinal lumen from which they are passed in the feces. The prepatent and patent periods vary, but most *Sarcocystis* species oocysts are first shed in the feces 7 to 14 days after ingesting sarcocysts and shedding occurs for weeks to months.

Sarcocysts, which are always located within a parasitophorous vacuole in the host cell cytoplasm, consist of a cyst wall that surrounds the metrocyte or the bradyzoites. The structure and thickness of the cyst wall differs among species of *Sarcocystis* and within each species as the sarcocyst matures.[60] Histologically, the sarcocyst wall may be smooth, striated or hirsute, or may possess complex branched protrusions. The structure of the sarcocyst wall is of taxonomic importance and used in species identification.[60] Internally, groups of zoites may be segregated into compartments by septa that originate from the sarcocyst wall or they may not be compartmentalized. Transmission electron microscopy of sarcocysts and PCR are often needed to identify sarcocysts to species.[60] Immunohistochemical tests are useful in experimental studies and are more helpful in locating schizonts in capillaries that sarcocysts in muscles. The presence of schizonts and only immature sarcocysts with metrocytes indicates acute or early infection and the presence of only mature sarcocysts indicates chronic infection.

### Clinical Sarcocystosis in Ruminants

*Sarcocystis* are generally nonpathogenic for the definitive host, and some species of *Sarcocystis* are also nonpathogenic for intermediate hosts (**Table 1**). Generally, species transmitted by canids are pathogenic, whereas those transmitted by felids are nonpathogenic. *S cruzi*, *Sarcocystis capracanis*, and *S tenella* are the most pathogenic species for cattle, goats, and sheep, respectively. Clinical signs are generally seen during the time that second generation schizonts are developing in blood vessels (acute phase). Three to 4 weeks after infection with a large dose of sporocysts (≥50,000), fever, anorexia, anemia, emaciation, and hair loss (particularly on the rump and tail in cattle) develop, and some animals may die. Pregnant animals may abort, and growth is slowed or arrested. Animals recover as sarcocysts begin to mature.

Dramatic gross lesions are seen in animals that die during the acute phase. Edema, hemorrhage, and atrophy of fat are commonly seen. The hemorrhages are most evident on the serosa of viscera, in cardiac and skeletal muscles, and in the sclera of the eyes. Hemorrhages vary from petechiae to ecchymoses several centimeters in diameter. Microscopic lesions may be seen in many organs and consist of necrosis, edema, and infiltrations of mononuclear cells. During the chronic phase, lesions are restricted to muscles and consist of nonsuppurative myositis and degeneration of sarcocysts.

### Zoonotic Sarcocystosis

Humans serve as the definitive host for *S hominis* and *S heydorni* of cattle and also serve as accidental intermediate hosts for several unidentified species of *Sarcocystis*.

| Intermediate Hosts | Sarcocystis Species | Sarcocyst Grossly Visible | Pathogenicity[a] | Definitive Hosts |
|---|---|---|---|---|
| Cattle (*Bos taurus*) | *S cruzi* | No, <1 mm | ++ | Dog, coyote, raccoon, red fox, wolf |
| | *S heydorni* | No, <1 mm | + | Human |
| | *S hirsuta* | Yes, ≤7 mm | + | Cat |
| | *S hominis* | Yes, ≤7 mm | + | Human, other primates |
| | *S rommeli* | No, <1 mm | ND | Cat |
| Sheep (*Ovis aries*) | *S arieticanis* | No, <1 mm | + | Dog |
| | *S gigantea* | Yes, ≤10 mm | - | Cat |
| | *S medusiformis* | Yes, ≤8 mm | - | Cat |
| | *S tenella* | No, <1 mm | ++ | Dog, coyote, red fox |
| Goat (*Capra hircus*) | *S capracanis* | No, <1 mm | ++ | Dog, coyote, red fox |
| | *S hircicanis* | Yes, ≤2.5 mm | ++ | Dog |
| | *S moule* | Yes, ≤7.5 mm | ND | Cat |
| Water Buffalo (*Bubalus bubalis*) | *S buffalonis* | Yes, ≤3 mm | ND | ND |
| | *S dubeyi* | No, <1 mm | ND | ND |
| | *S fusiformis* | Yes, ≤3 mm | - | Cat |
| | *S levinei* | No, <1 mm | - | Dog |

**Table 1**
**Common species of *Sarcocystis* in ruminants**

[a] ++ = Pathogenic, + = moderately pathogenic, - = not pathogenic, ND = not determined.

Intestinal sarcocystosis is acquired by ingesting uncooked beef containing sarcocysts of *S hominis* symptoms include nausea, stomachache and abdominal pain. Sporocysts are shed 11 to 13 days after ingesting the infected beef.[60]

### Eosinophilic Myositis

Eosinophilic myositis (EM) is a specific inflammatory condition of striated muscles, mainly owing to accumulations of eosinophils.[60,61] It has been found mainly in cattle and occasionally in sheep. The affected animals are usually clinically normal and EM lesions are discovered at meat inspection after slaughter. Gross lesions consist of green to pale yellow areas that may be up to 15 cm long. The pathogenesis of EM is not clear and EM lesions have never been found in livestock species experimentally infected with *Sarcocystis* species.[60] Degenerating sarcocysts have been found in sections of lesions of EM,[61] but the high prevalence of *Sarcocystis* spp. infection in naturally infected cattle with no EM makes it difficult to designate *Sarcocystis* as the cause of EM in cattle.

Condemnation of beef containing lesions of EM or grossly visible sarcocysts (*S hirsuta*) can be a serious economic problem.[62,63] In a study, 974 of 1,622,402 cattle (0.06%) slaughtered in 1965 to 1966 in the United States were condemned because of EM.[63] In another report 18 bovine carcasses from 1 slaughter plant in the United States were condemned because of grossly visible *S hirsuta* sarcocysts.[62]

### Diagnosis

The antemortem diagnosis of muscular sarcocystosis can only be made by histologic examination of muscle collected by biopsy or at necropsy.[60] The finding of immature sarcocysts with metrocytes suggests recently acquired infection but if only mature sarcocysts are present then the infection is chronic.[60]

An inflammatory response associated with sarcocysts may help to distinguish an active disease process from incidental finding of sarcocysts. There are several serologic tests and PCR techniques developed experimentally to distinguish *Sarcocystis* species in ruminants but there are pitfalls to serologic and molecular diagnosis of sarcocystosis in animals and none are commercially available.[63]

The diagnosis of intestinal *Sarcocystis* infection in a definitive host can be made by is by fecal examination. As has been mentioned, sporocysts or oocysts of *Sarcocystis* are shed fully sporulated in feces whereas those of *N caninum*, *T gondii*, and *Cystoisospora spp.* are shed unsporulated. It is not possible to distinguish one species of *Sarcocystis* from another by the structure of sporocysts in the feces. PCR on sporocysts can be used to determine the species present in definitive hosts.

### Epidemiology and Control

*Sarcocystis* infection is common in ruminants worldwide.[60] Several factors contribute to the high prevalence in muscular infections in ruminants. Several species may infect a particular host and there maybe abundant definitive hosts for each species infecting that host. Each infected definitive host can shed millions of infectious sporocysts over several months contaminating the environment. *Sarcocystis* sporocysts and oocysts remain viable for many months in the environment, are resistant to freezing, and can overwinter on pasture. Oocysts and sporocysts are spread by invertebrate transport hosts to other areas. The definitive host develops little or no immunity, and repeat shedding of sporocysts occurs each time a meal of infected meat is consumed. *Sarcocystis* oocysts, unlike those of many other species of coccidia, are passed in feces in the infective form freeing them from dependence on warm moist weather conditions for maturation to infectivity.[60]

There is no vaccine to protect ruminants against sarcocystosis. Shedding of *Sarcocystis* oocysts and sporocysts in feces of the definitive hosts is the key factor in the spread of *Sarcocystis* infection; to interrupt this cycle, carnivores should be excluded from animal houses and from feed, water and bedding for livestock. Uncooked meat or offal should never be fed to carnivores. Because freezing can drastically decrease or eliminate infectious sarcocysts, meat should be frozen if not cooked. Exposure to heat at 55°C for 20 minutes kills sarcocysts.[64] Dead livestock should be buried or incinerated. Dead animals should never be left in the field for vultures and carnivores to eat.

### SUMMARY

Much needs to be learned about preventing *N caninum*, *T gondii*, and *Sarcocystis* spp. infections in ruminants. Additional research on inducing immunity to congenital transmission of *N caninum* is needed but few laboratories are exploring this difficult area of study. In addition, research is needed regarding the pathogenesis of *N caninum* abortion, life cycle of the parasite in cattle, and sources of infection. *T gondii* abortions in sheep and goats remain a production challenge to the industry. The effects of *Sarcocystis* infections in ruminants are difficult to evaluate because nearly all ruminants raised on pasture contain sarcocysts in their muscles.

### DISCLOSURE

The authors have nothing to disclose.

## REFERENCES

1. Dubey JP, Hemphill A, Calero-Bernal R, et al. Neosporosis in animals. 1st edition. Boca Raton (FL): CRC Press, Taylor & Francis Group; 2017. p. 1–529. ISBN: 9781498752541.
2. Bjerkås I, Mohn SF, Presthus J. Unidentified cyst-forming sporozoon causing encephalomyelitis and myositis in dogs. Z Parasitenkd 1984;70:271–4.
3. Dubey JP, Carpenter JL, Speer CA, et al. Newly recognized fatal protozoan disease of dogs. J Am Vet Med Assoc 1988;192:1269–85.
4. Dubey JP, Hattel AL, Lindsay DS, et al. Neonatal *Neospora caninum* infections in dogs: isolation of the causative agent and experimental transmission. J Am Vet Med Assoc 1988;193:1259–63.
5. Lindsay DS, Dubey JP. Immunohistochemical diagnosis of *Neospora caninum* in tissue sections. Am J Vet Res 1989;50:1981–3.
6. McAllister MM, Dubey JP, Lindsay DS, et al. Dogs are definitive hosts of *Neospora caninum*. Int J Parasitol 1998;28:1473–8.
7. Lindsay DS, Dubey JP, Duncan RB. Confirmation that the dog is a definitive host for *Neospora caninum*. Vet Parasitol 1999;82:327–33.
8. Gondim LFP, McAllister MM, Pitt WC, et al. Coyotes (*Canis latrans*) are definitive hosts of *Neospora caninum*. Int J Parasitol 2004;34:159–61.
9. Dubey JP, Jenkins MC, Rajendran C, et al. Gray wolf (*Canis lupus*) is a natural definitive host for *Neospora caninum*. Vet Parasitol 2017;181:382–7.
10. Rosypal AC, Lindsay DS. The sylvatic cycle of *Neospora caninum*: where do we go from here? Trends Parasitol 2005;25:439–40.
11. Dubey JP, Lindsay DS. A review of *Neospora caninum* and neosporosis. Vet Parasitol 1996;67:1–59.
12. Ortega-Mora LM, Ferre I, del Pozo I, et al. Detection of *Neospora caninum* in semen of bulls. Vet Parasitol 2003;117:301–8.
13. Baillargeon P, Fecteau G, Paré J, et al. Evaluation of the embryo transfer procedure proposed by the International Embryo Transfer Society as a method of controlling vertical transmission of *Neospora caninum* in cattle. J Am Vet Med Assoc 2001;218:1803–6.
14. Davison HC, Guy CS, McGarry JW, et al. Experimental studies on the transmission of *Neospora caninum* between cattle. Res Vet Sci 2001;70:163–8.
15. Uggla A, Stenlund S, Holmdahl OJM, et al. Oral *Neospora caninum* inoculation of neonatal calves. Int J Parasitol 1998;28:1467–72.
16. Dijkstra T, Eysker M, Schares G, et al. Dogs shed *Neospora caninum* oocysts after ingestion of naturally infected bovine placenta but not after ingestion of colostrum spiked with *Neospora caninum* tachyzoites. Int J Parasitol 2001;31:747–52.
17. Thilsted JP, Dubey JP. Neosporosis-like abortions in a herd of dairy cattle. J Vet Diagn Invest 1989;1:205–9.
18. Anderson ML, Blanchard PC, Barr BC, et al. *Neospora*-like protozoan infection as a major cause of abortion in California dairy cattle. J Am Vet Med Assoc 1991; 198:241–4.
19. McAllister M, Huffman EM, Hietala SK, et al. Evidence suggesting a point source exposure in an outbreak of bovine abortion due to neosporosis. J Vet Diagn Invest 1996;8:355–7.
20. McAllister MM, Björkman C, Anderson-Sprecher R, et al. Evidence of point-source exposure to *Neospora caninum* and protective immunity in a herd of beef cows. J Am Vet Med Assoc 2000;217:881–7.

21. Anderson ML, Palmer CW, Thurmond MC, et al. Evaluation of abortions in cattle attributable to neosporosis in selected dairy herds in California. J Am Vet Med Assoc 1995;207:1206–10.

22. Okeoma CM, Williamson NB, Pomroy WE, et al. Isolation and molecular characterization of *Neospora caninum* in cattle in New Zealand. N Z Vet J 2004;52:364–70.

23. Sawada M, Kondo H, Tomioka Y, et al. Isolation of *Neospora caninum* from the brain of a naturally infected adult dairy cow. Vet Parasitol 2000;90:247–52.

24. Okeoma CM, Williamson NB, Pomroy WE, et al. The use of PCR to detect *Neospora caninum* DNA in the blood of naturally infected cows. Vet Parasitol 2004;122:307–15.

25. Dubey JP, Schares G. Diagnosis of bovine neosporosis. Vet Parasitol 2006;140:1–34.

26. Dubey JP, Buxton D, Wouda W. Pathogenensis of bovine neosporosis. J Comp Pathol 2006;134:267–89.

27. Álvarez-García G, Collantes-Fernández E, Costas E, et al. Influence of age and purpose for testing on the cut-off selection of serological methods in bovine neosporosis. Vet Res 2003;34:341–52.

28. Baszler TV, Gay LJC, Long MT, et al. Detection by PCR of *Neospora caninum* in fetal tissues from spontaneous bovine abortions. J Clin Microbiol 1999;37:4059–64.

29. von Blumröder D, Schares G, Norton R, et al. Comparison and standardisation of serological methods for the diagnosis of *Neospora caninum* infection in bovines. Vet Parasitol 2004;120:11–22.

30. Schares G, Conraths FJ, Reichel MP. Bovine neosporosis: comparison of serological methods using outbreak sera from a dairy herd in New Zealand. Int J Parasitol 1999;29:1659–67.

31. Trees AJ, Williams DJL. Endogenous and exogenous transplacental infection in *Neospora caninum* and *Toxoplasma gondii*. Trends Parasitol 2005;21:558–61.

32. Björkman C, McAllister MM, Frössling J, et al. Application of the *Neospora caninum* IgG avidity ELISA in assessment of chronic reproductive losses after an outbreak of neosporosis in a herd of beef cattle. J Vet Diagn Invest 2003;15:3–7.

33. Baszler TV, Adams S, Vander-Schalie J, et al. Validation of a commercially available monoclonal antibody-based competitive-inhibition enzyme-linked immunosorbent assay for detection of serum antibodies to *Neospora caninum* in cattle. J Clin Microbiol 2001;39:3851–7.

34. Reichel MP, Ellis JT. Control options for *Neospora caninum* infections in cattle - current state of knowledge. N Z Vet J 2002;50:86–92.

35. Dijkstra T, Barkema HW, Hesselink JW, et al. Point source exposure of cattle to *Neospora caninum* consistent with periods of common housing and feeding and related to the introduction of a dog. Vet Parasitol 2002;105:89–98.

36. Schares G, Bärwald A, Staubach C, et al. Potential risk factors for bovine *Neospora caninum* infection in Germany are not under the control of the farmers. Parasitology 2004;129:301–9.

37. Innes EA, Andrianarivo AG, Björkman C, et al. Immune responses to *Neospora caninum* and prospects for vaccination. Trends Parasitol 2002;18:497–504.

38. Marugan-Hernandez V. *Neospora caninum* and bovine neosporosis: current vaccine research. J Comp Pathol 2017;157:193–200.

39. Barling KS, Lunt DK, Graham SL, et al. Evaluation of an inactivated *Neospora caninum* vaccine in beef feedlot steers. J Am Vet Med Assoc 2003;222:624–7.

40. Romero JJ, Pérez E, Frankena K. Effect of a killed whole *Neospora caninum* ta-chyzoite vaccine on the crude abortion rate of Costa Rican dairy cows under field conditions. Vet Parasitol 2004;23:149–59.
41. Marsh AE, Barr BC, Packham AE, et al. Description of a new *Neospora* species (Protozoa: Apicomplexa: Sarcocystidae). J Parasitol 1998;84:983–91.
42. Marsh AE, Howe DK, Wang G, et al. Differentiation of *Neospora hughesi* from *Neospora caninum* based on their immunodominant surface antigen, SAG1 and SRS2. Int J Parasitol 1999;29:1575–82.
43. Walsh CP, Vemulapalli R, Sriranganathan N, et al. Molecular comparison of the dense granule proteins GRA6 and GRA7 of *Neospora hughesi* and *Neospora caninum*. Int J Parasitol 2001;31:253–8.
44. Walsh CP, Duncan RB, Zajac AM, et al. *Neospora hughesi*: experimental infec-tions in mice, gerbils, and dogs. Vet Parasitol 2000;92:119–28.
45. Dubey JP. Toxoplasmosis of animals and man. 2nd edition. Boca Raton (FL): CRC Press; 2010.
46. Dubey JP, Lindsay DS, Speer CA. Structure of *Toxoplasma gondii* tachyzoites, bradyzoite, and sporozoites, and biology and development of tissue cysts. Clin Microbiol Rev 1998;11:267–99.
47. Dubey JP, Frenkel JK. Cyst-induced toxoplasmosis in cats. J Protozool 1972;19: 155–77.
48. Speer CA, Dubey JP. Ultrastructural differentiation of *Toxoplasma gondii* schiz-onts (types B to E) and gamonts in the intestines of cats fed bradyzoites. Int J Parasitol 2005;35:193–206.
49. Dubey JP. Unexpected oocyst shedding by cats fed *Toxoplasma gondii* tachy-zoites: in vivo stage conversion and strain variation. Vet Parasitol 2005;133: 289–98.
50. Dubey JP. Comparative infectivity of oocysts and bradyzoites of *Toxoplasma gon-dii* for intermediate (mice) and definitive (cats) hosts. Vet Parasitol 2006;40: 69–75.
51. Wallace GD. Serologic and epidemiologic observations on toxoplasmosis on three Pacific Atolls. Am J Epidemiol 1969;90:103–11.
52. Munday B. Serologic evidence for *Toxoplasma* infection in isolated groups of sheep. Res Vet Sci 1972;13:100–2.
53. Dubey JP, Rollor EA, Smith K, et al. Low seroprevalence of *Toxoplasma gondii* in feral pigs from a remote island lacking cats. J Parasitol 1997;83:839–41.
54. Dubey JP. Toxoplasmosis - a waterborne zoonosis. Vet Parasitol 2004;26:57–72.
55. Tenter AM, Heckeroth AR, Weiss LM. *Toxoplasma gondii*: from animals to hu-mans. Int J Parasitol 2000;30:1217–58.
56. Desmonts G, Couvreur J. Congenital toxoplasmosis. A prospective study of 378 pregnancies. N Engl J Med 1974;290:1110–6.
57. Remington JS, McLeod R, Thulliez P, et al. Toxoplasmosis. In: Remington JS, Klein JO, editors. Infectious diseases of the fetus and newborn infant. Philadel-phia: W. B. Saunders; 2001. p. 205–346.
58. Lindsay DS, Dubey JP. *Toxoplasma gondii*: the changing paradigm of congenital toxoplasmosis. Parasitology 2011;138:1829–31.
59. Buxton D. Toxoplasmosis: the first commercial vaccine. Parasitol Today 1993;9: 335–7.
60. Dubey JP, Calero-Bernal R, Rosenthal BM, et al. Sarcocystosis of animals and man. 2nd edition. Boca Raton (FL): CRC Press; 2016.
61. Wouda W, Snoep JJ, Dubey JP. Eosinophilic myositis due to *Sarcocystis hominis* in a beef cow. J Comp Pathol 2006;135:249–53.

62. Dubey JP, Udtujan RM, Cannon L, et al. Condemnation of beef because of *Sarcocystis hirsuta* infection. J Am Vet Med Assoc 1990;196:1095–6.
63. Tenter AM. Current research on *Sarcocystis* species of domestic animals. Int J Parasitol 1995;25:1311–30.
64. Fayer R. Effects of refrigeration, cooking, and freezing on *Sarcocystis* in beef from retail food stores. Proc Helminthol Soc Wash 1975;42:138–40.