

# Neosporosis, Toxoplasmosis, and Sarcocystosis in Ruminants

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## Neosporosis

### *Etiologic agent*

Neosporosis is caused by the protozoan parasite, *Neospora caninum*. Until 1988, *N caninum* was confused with a closely related parasite, *Toxoplasma gondii* [1,2]. Since its first recognition as a clinical disease of dogs in Norway in 1984 [3], neosporosis has emerged as a serious disease of cattle and dogs worldwide [4–6]. Additionally, clinical neosporosis has been reported occasionally in other animals, and antibodies to *N caninum* have been found in the sera of many species of domestic and free-range land mammals as well as in marine mammals [7]. Until now, however, viable *N caninum* has been isolated only from dogs, cattle, white-tailed deer, water buffaloes, and sheep [7]. At present, there is no evidence that *N caninum* infects human beings.

*N caninum* is a coccidian parasite, and its oocysts have been found in feces of dogs and coyotes [8–13]. Thus, dogs are the intermediate and definitive host for *N caninum*. The life cycle is typified by three infectious stages: tachyzoites, tissue cysts, and oocysts (Fig. 1). Tachyzoites and tissue cysts are the stages found in the intermediate hosts, and they occur intracellularly [2]. Tachyzoites are approximately 6  $\mu\text{m}$   $\times$  2  $\mu\text{m}$ . Tissue cysts are often round or oval in shape, up to 107  $\mu\text{m}$  long, and are found primarily in

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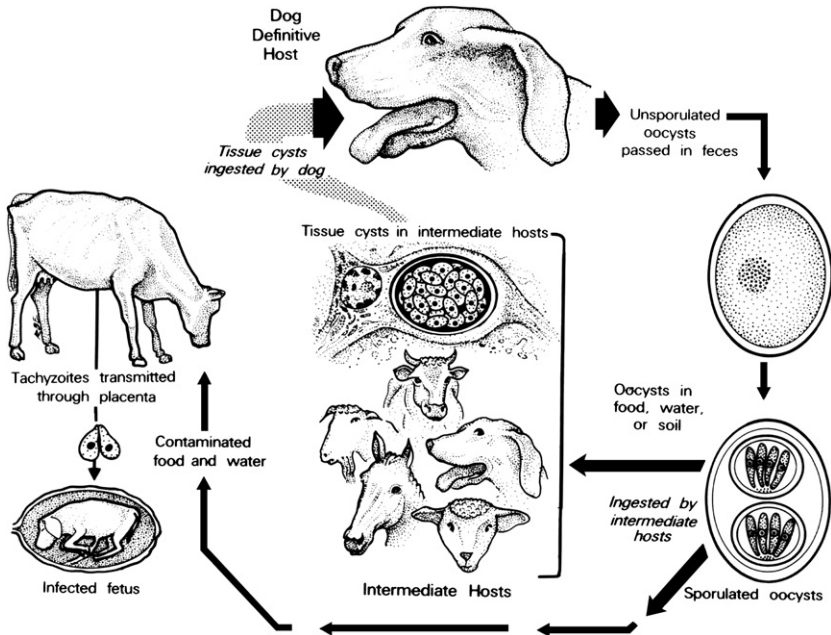


Fig. 1. Life cycle of *Neospora caninum*. (From Dubey JP. Review of *Neospora caninum* and neosporosis in animals. Korean J Parasitol 2003;41:1–16.)

the central nervous system (CNS). The tissue cyst wall is up to 4  $\mu\text{m}$  thick, and the enclosed bradyzoites are 7 to 8  $\mu\text{m} \times 2 \mu\text{m}$ .

*N. caninum* oocysts are excreted unsporulated in feces and measure approximately 12  $\mu\text{m}$  in diameter, and sporulation occurs outside the host. At present, little is known regarding the frequency of shedding of oocysts, the survival of the oocysts in the environment, and whether other canids are also definitive hosts for *N. caninum* [7]. The parasite can be transmitted transplacentally in several hosts, and the vertical route is the major mode of its transmission in cattle [14–16]. There is no cow-to-cow transmission of *N. caninum*. Although most *N. caninum* infections in cattle are transmitted transplacentally, postnatal rates have been variable depending on the region of the country, type of test used, and cutoff values used [7]. Although *N. caninum* has been found in bovine semen [17], it is unlikely that *N. caninum* is transmitted venereally or by embryo transfer from the donor cows. Actually, embryo transfer is even recommended as a method of control to prevent vertical transmission [18]. Nevertheless, it is prudent to test all recipients, and embryos should not be transferred to seropositive cows. Lacrogenic transmission of *N. caninum* is considered unlikely [19–21]. Carnivores can acquire infection by ingestion of infected tissues.

## Neosporosis in cattle

### *Clinical signs*

*N. caninum* causes abortion in dairy and beef cattle [5,22–28]. Cows of any age may abort from 3 months of gestation to 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 dairy and beef cattle. Up to 95% of calves born congenitally infected from seropositive dams remain clinically normal, however. The age of the dam, lactation number, and history of abortion generally do not affect the rate of congenital infection, but there are reports indicating that vertical transmission is more efficient in younger than older cows in persistently infected cattle [16]. The infected replacement heifers may abort or transplacentally infect their offspring.

Clinical signs have only been reported in cattle younger than 2 month of age [5]. *N. caninum*-infected calves may have neurologic signs, be underweight, be unable to rise, or be born without clinical signs of disease. Hind limbs, 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 an asymmetric appearance in the eyes. *N. caninum* occasionally causes birth defects, including hydrocephalus and narrowing of the spinal cord [5].

Abortions may be epidemic or endemic [22–28]. Up to 33% of dairy cow fetuses have been reported to abort within a few months. Abortions are considered epidemic if more than 10% of cows at risk aborted within 6 to 8 weeks. A small proportion (<5%) of cows have been reported to have repeated abortion because of neosporosis [25]. Cows with *N. caninum* antibodies (seropositive) are more likely to abort than seronegative cows. There is a rise in antibody titers 4 to 5 months before parturition. These observations strongly suggest reactivation of latent infection. Little is known of the mechanism of reactivation. It is likely that there is parasitemia during pregnancy, leading to fetal infection. *N. caninum* has never been identified in histologic sections taken from adult cows, however, whereas viable *N. caninum* has been isolated from the brain of only two cows [29,30]. Although it is reasonable to speculate that pregnancy-induced immune suppression or hormonal imbalance may reactivate latent tissue cysts of *N. caninum*, such a mechanism has not been demonstrated for neosporosis. *N. caninum* DNA has been found in blood of naturally infected cattle, indicating parasitemia [31]. *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 and seroprevalences for each host were tabulated recently [7]. Quantitative 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* [7].

Serologic prevalence in cattle varies, depending on the country, region, type of serologic test used, and cutoff level used to determine the exposure. In some dairies, up to 87% of cows are seropositive [5,7].

In general, less is known about the causes of abortion in beef cattle than in dairy cattle because of the difficulty in finding small fetuses expelled in the first trimester. Therefore, there are no accurate assessments of *Neospora*-induced losses in beef cattle. Because clinical disease has not been reported in calves older than 2 months of age, there is no direct evidence of *N caninum*-associated morbidity in adult cattle.

### 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 neosporosis [32]. 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 [33]. Although lesions of neosporosis are found in several organs, fetal brain is the most consistently affected organ [33]. 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 (H&E)-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 H&E-stained sections. The most characteristic lesion of neosporosis is focal encephalitis characterized by necrosis and nonsuppurative inflammation [33]. Hepatitis is more common in epizootic than sporadic abortions [34]. Lesions are also present in the placenta but protozoa are difficult to find.

The efficiency of the diagnosis by polymerase chain reaction (PCR) is dependent on the laboratory, stage of the autolysis of the fetus, and sampling procedures [32,35,36]. Although immunohistochemical demonstration of *N caninum* in lesions is the best evidence for the etiology of abortion at the present time, it is quite insensitive. *N caninum* DNA can be detected by PCR in formalin-fixed paraffin-embedded bovine aborted brain tissue.

Several serologic tests can be used to detect *N caninum* antibodies, including various ELISAs, the indirect fluorescent antibody test (IFAT), and the *Neospora* agglutination test (NAT) [37–48]. There are several modifications of the ELISA to detect antibodies to *N caninum* in sera or milk using whole parasite, whole parasite lysate, purified proteins, recombinant proteins, and

tachyzoite proteins absorbed on immune stimulating complexes (ISCOM) particles, and some of these tests were compared recently in a multicenter study in various laboratories in Europe [46]. Avidity ELISAs designed to distinguish recent and chronic infections in cattle seem to be promising to distinguish endemic and epidemic abortion [45]. 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 [45]. Another modification of the ELISA is the antigen-capture ELISA (cELISA). This test detects (captures) a 65-kd antigen in sera of infected cattle using a specific monoclonal antibody, and this test is commercially available [47,48]. Immunoblots are useful in detecting *N caninum*-specific antibodies [32].

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 is dependent on the stage of gestation, level of exposure, and time between infection and abortion. Immunoblotting using *N caninum*-specific antigen improves diagnosis. Although blood serum or any body fluid from the fetus may be used for serologic diagnosis, peritoneal fluid is better than other body fluids. In calves, presuckling 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 bovine species 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 are dependent on antigen composition, secondary antibodies, and other reagents [32]. In addition, cutoff 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 a cutoff level. Although *N caninum* is closely related to *T gondii*, *Sarcocystis* species, and other apicomplexans, cross-reactivity has not been a major issue. In general, antibody titers are higher in cattle that have aborted because of neosporosis than in those with a 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. Therefore, culling is one way at present to prevent this transmission from cow to heifer [49,50]. Culling is not practical if the prevalence of *N caninum* in a herd is extremely high, however. Before making a decision to cull, it is advisable to estimate the prevalence of *N caninum* in the herd. Bulk milk testing can provide the preliminary information about the presence 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

into the transmission of *N caninum* in a given herd. In herds with high transplacental transmission, the prevalence of *N caninum* in cattle of different ages is about the same and there is a high correlation between infection in dams and daughters. To reduce 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 two of the methods that can be adapted. Drugs that kill encysted *N caninum* in bovine tissues are unknown.

To prevent horizontal (from outside sources) transmission, it is important to prevent exposure of the cows to feed and water contaminated with oocysts [51–53]. Dogs and other canids should not be allowed in cattle barns or pasture, although this is not always easy to achieve. How dogs become infected with *N caninum* is not known. 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 [21]. Little is known at present regarding the frequency of shedding of *N caninum* oocysts by canids in nature, the resistance of the oocysts, and whether dogs shed oocysts more than once. Until more definitive hosts of *N caninum* are found, dogs and coyotes should not be allowed to eat aborted fetuses, fetal membranes, or dead calves. Other factors, such as farm location, can also be a risk [53]. Drugs that prevent transmission of the parasite from the dam to the fetus are unknown, but research is continuing in this area.

There is evidence that cattle can develop protective immunity to subsequent neosporosis abortion [54–57]. 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 persistent infection [56]. Therefore, to elicit protective immunity against abortion in cows that already harbor a latent infection is a problem.

Currently, there is a killed-parasite commercial *N caninum* vaccine (Neo Guard), but there are no convincing data about the efficiency of this vaccine to prevent *N caninum*-associated abortion in cattle [58,59].

### 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, a 16-day-old rhinoceros (*Ceratotherium simum*), a juvenile raccoon (*Procyon lotor*), a 2-month-old black-tailed deer (*Odocoileus hemionus columbianus*), 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*) [7]. A new species, *Neospora*

*hughesi*, has been described in horses [60]. It is uncertain whether *N caninum* infects horses.

## Toxoplasmosis

### Etiologic agent

Toxoplasmosis is caused by the infection with the protozoan *T gondii* [61]. It is among the most common parasites of animals, and *T gondii* is the only known species. Felids are the definitive hosts, and warm-blooded animals are intermediate hosts [62]. There are three infectious stages of *T gondii* for all hosts: tachyzoites (individually and in groups), bradyzoites (in tissue cysts), and sporozoites (in oocysts) (Fig. 2).

The tachyzoite is often crescent shaped and  $2\ \mu\text{m} \times 6\ \mu\text{m}$  in size. It enters the host cell by active penetration of the cell membrane and becomes surrounded by a parasitophorous vacuole that protects it from host defense mechanisms. The tachyzoite multiplies asexually by repeated binary divisions until the host cell ruptures. After an unknown numbers of divisions, *T gondii* tachyzoites give rise to another stage called a tissue cyst. Tissue cysts grow and remain intracellular. They vary in size from 5 to 70  $\mu\text{m}$  and contain a few to several hundred bradyzoites [63]. Although tissue cysts may develop in visceral organs, including the lungs, liver, and kidneys, they are more prevalent

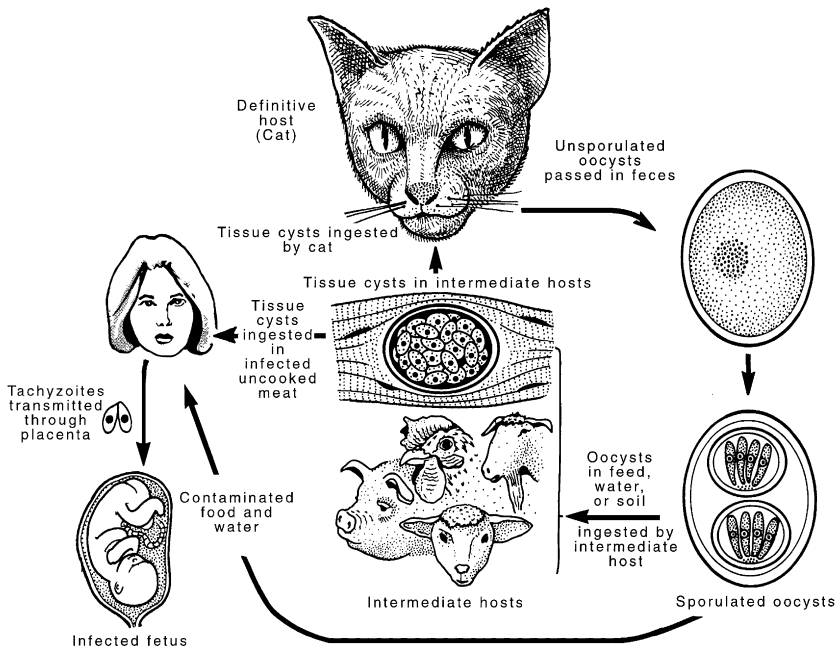


Fig. 2. Life cycle of *Toxoplasma gondii*. (From Dubey JP. Toxoplasmosis—a waterborne zoonosis. *Vet Parasitol* 2004;126:57–72.)



in muscular and neural tissues, including the brain, eye, and skeletal and cardiac muscle. Intact tissue cysts are probably harmless and can persist for the life of the host [63]. The tissue cyst wall is elastic and thin ( $<0.5 \mu\text{m}$ ), and it may enclose hundreds of crescent-shaped slender bradyzoites, each measuring  $7 \mu\text{m} \times 1.5 \mu\text{m}$ . Bradyzoites differ only slightly from tachyzoites in having a nucleus situated toward the posterior end, whereas the nucleus in tachyzoites is more central. Additionally, bradyzoites are more slender than tachyzoites and less susceptible to destruction by proteolytic enzymes.

On ingestion by cats, the wall of the tissue cyst is digested by the proteolytic enzymes in the stomach and small intestine and bradyzoites are released. Some penetrate the lamina propria of the intestine and multiple as tachyzoites. Within a few hours, *T. gondii* may disseminate to extraintestinal tissues. Other bradyzoites penetrate epithelial cells of the small intestine and initiate development of numerous generations of asexual (types A–E) schizonts [64,65]. The organisms (merozoites) released from schizonts form male and female gametes. After the female gamete is fertilized by the male gamete, oocyst wall formation begins around the fertilized gamete. When oocysts are mature, they are discharged into the intestinal lumen by the rupture of intestinal epithelial cells. *T. gondii* persists in the intestinal and extraintestinal tissue of cats for at least several months, and possibly for the life of the cat.

Oocysts of *T. gondii* are formed only in cats, including domestic and wild felids. Cats shed oocysts after ingesting tachyzoites, bradyzoites, or sporozoites [62,64,66–70]. Less than 50% of cats shed oocysts after ingesting tachyzoites or oocysts, however, whereas nearly all shed oocysts after ingesting tissue cysts [66,68–70].

Oocysts in freshly passed feces are unsporulated (noninfective), subspherical to spherical in shape, and  $10 \mu\text{m} \times 12 \mu\text{m}$  in diameter. Sporulation occurs outside the cat and within 1 to 5 days depending on aeration and temperature. Sporulated oocysts contain two ellipsoidal sporocysts. Each sporocyst contains four sporozoites. The sporozoites are  $2 \mu\text{m} \times 6$  to  $8 \mu\text{m}$  in size.

Hosts, including felids, can acquire *T. gondii* by ingesting tissues of infected animals or food or drink contaminated with sporulated oocysts or by transplacental transmission. After ingestion, bradyzoites released from tissue cysts or sporozoites from oocysts penetrate intestinal tissues, transform to tachyzoites, multiply locally, and are disseminated in the body via blood or lymph. After a few multiplication cycles, tachyzoites give rise to bradyzoites in a variety of tissues. *Toxoplasma gondii* infection during pregnancy can lead to infection of the fetus. Congenital toxoplasmosis in human beings, sheep, and goats can kill the fetus.

After ingestion of sporulated oocysts, sporozoites excyst, penetrate enterocytes and goblet cells of the intestinal epithelium, and are carried to the lamina propria via an unknown mechanism. Some sporozoites can be found circulating in peripheral blood as early as 4 hours after ingestion. Most remain in the lamina propria, however, where they multiply in a variety of cells, including vascular endothelium, fibroblasts, mononuclear cells, and



segmented leukocytes, but not in erythrocytes. Edema, necrosis of the lamina propria, and sloughing of the intestinal mucosa can produce severe enteritis. Infection can eventually spread to all other organs.

### *Host-parasite relation*

*T gondii* can multiply in most mammalian cells. How it is 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 in the immune-mediated destruction of *T gondii*.

Immunity does not eliminate an established infection. *T gondii* tissue cysts persist several years after acute infection. The ultimate fate of tissue cysts is not fully known. Some may rupture during the life of the host, and the released bradyzoites may be destroyed by the host's immune responses. In immunosuppressed individuals, however, infection can be reactivated by dissemination of bradyzoites and conversion to tachyzoites.

The pathogenicity of *T gondii* is determined by many factors, including the susceptibility of the host species, virulence of the parasitic strain, and stage. Oocyst-induced infections are the most clinically severe in intermediate hosts, and this is not dose dependent [61]. *T gondii* isolates differ remarkably in their virulence to outbred mice, but virulence of *T gondii* in mice should not be equated with virulence in human beings or domestic animals. *T gondii* has also adapted to an oocyst-oral cycle in herbivores (intermediate hosts) and to a tissue cyst-oral cycle in carnivores, especially in the cat. *T gondii* oocysts are less infective and less pathogenic for the cat than for mice [67,70]. For example, 1 live oocyst is orally infective to mice and pigs [71], whereas 100 or more oocysts may be required to establish infection in a cat [70]. The reverse may be true for bradyzoites. By mouth, bradyzoites are less infective to mice than cats [72]. Cats can shed millions of oocysts after ingesting as few as 1 bradyzoite, whereas 100 bradyzoites may not be infective to mice by the oral route [70,72]. Although *T gondii* can be transmitted orally by ingesting tissue cysts, epidemiologic evidence indicates that cats are essential in perpetuation of the life cycle, because *T gondii* infection is rare or absent in areas devoid of cats [73–75].

### *Epidemiology*

Domestic cats are probably the major source of contamination, because they are common and produce large numbers of *T gondii* oocysts [64,72]. Sporulated oocysts survive for long periods under moderate environmental conditions. For example, they can survive in moist soil for months to years [61]. Oocysts in soil can be spread mechanically by flies, cockroaches, dung beetles, and earthworms. Oocysts are known to survive on fruits and vegetables for long periods [76]. Humans may acquire toxoplasmosis by petting dogs that have rolled over in infected cat feces [77–79].

Although only a few cats may be shedding *T gondii* oocysts at any given time, the enormous numbers produced and their resistance to destruction ensure widespread contamination [80]. Latently infected cats can shed oocysts after challenge infection. Congenitally infected kittens can also excrete oocysts. Infection rates in cats are largely determined by the rate of infection in the local avian and rodent populations that serve as a food source. For epidemiologic surveys, seroprevalence data for cats are more useful than results of fecal examination, because cats with antibodies have probably already shed oocysts and are an indicator of environmental contamination [64].

Infection in human beings is probably most often the result of ingestion of tissue cysts contained in raw or undercooked meat, because *T gondii* is common in many animals used for food, including sheep, pigs, and rabbits. Viable *T gondii* has not been isolated from beef, and the role of cattle in the transmission of infection to human beings is at best uncertain [81]. Although the prevalence of *T gondii* in pigs raised under good management practices has decreased drastically [81], infection rates can be high in pigs raised outdoors and in unhygienic conditions [82]. Tissue cysts can survive in food animals for years [61]. Virtually all edible portions of carcasses can be infected with viable *T gondii*.

Cultural habits may also affect the acquisition of *T gondii* infection. The high incidence of *T gondii* infection in human beings in France seems to be related, in part, to the French habit of eating some meat raw. In contrast, the high prevalence of the infection in Central America and South America is in probably attributable to high levels of contamination of the environment by oocysts [83]. Outbreaks of acute toxoplasmosis have been reported in human beings by drinking water contaminated with oocysts [84,85]. It should be noted, however, that the relative frequency of acquisition of toxoplasmosis from eating raw meat and that attributable to ingestion of food contaminated by oocysts from cat feces in the general population are unknown. At present, there are no tests to distinguish oocyst- versus meat-acquired *T gondii* infection.

In addition to infection as a result of ingestion of oocysts and by eating infected raw meat, transmission of toxoplasmosis can be by semen transfusion, by ingestion of milk or saliva, and by eating eggs. The stages most likely to be involved in these transmissions are tachyzoites, which are not environmentally resistant and are killed by water. The probability of transmission of *T gondii* by these means is rare, however. There is little if any danger of *T gondii* infection by drinking cow's milk, which, in any case, is generally pasteurized or even boiled, but infection has followed drinking unboiled goat's milk. Raw hens' eggs, although an important source of *Salmonella* infection, are extremely unlikely to harbor *T gondii*. Transmission by sexual activity, including kissing, is probably rare and epidemiologically unimportant.

Transmission can also occur through blood transfusions and organ transplants, with transplantation being the more important; this is a recent development. In people undergoing transplantation, toxoplasmosis may arise

from implantation of an organ or bone marrow from an infected donor into a nonimmune immunocompromised recipient or from induction of disease in an immunocompromised latently infected recipient. The tissue cysts in the transplanted tissue or in the latently infected person are probably the source of the infection. In both cases, the cytotoxic and immunosuppressive therapy given to the recipient is the cause of the induction of the active infection and the disease.

### *Clinical toxoplasmosis*

*T gondii* is capable of causing severe disease in animals other than human beings [61] and is responsible for great losses to the livestock industry. In sheep and goats, it may cause embryonic death and resorption, fetal death and mummification, abortion, stillbirth, and neonatal death. Disease is more severe in goats than in sheep. Outbreaks of toxoplasmosis in pigs have been reported from several countries, especially Japan, and mortality is more common in young pigs than in adult pigs. Pneumonia, myocarditis, encephalitis, and placental necrosis occur in infected pigs. Cattle and horses are more resistant to clinical toxoplasmosis than are other species of livestock; there is no confirmed report of clinical toxoplasmosis in cattle, horses, and water buffaloes. In cats and dogs, the disease is most severe in young animals. Common clinical manifestations of canine toxoplasmosis are respiratory distress, ataxia, and diarrhea. In most infected dogs, pneumonia is caused by a combination of *T gondii* and distemper virus, because the virus is immunosuppressive. Respiratory distress is a common clinical sign in cats with toxoplasmosis. Although cats of any age can die of toxoplasmosis, kittens and those with depressed immunity are the most likely victims [86,87].

Sporadic and widespread outbreaks of toxoplasmosis occur in rabbits, mink, birds, and other domesticated and wild animals [61,88–91]. Toxoplasmosis is severe in many species of Australian marsupials and in marsupials, New World monkeys, Pallas cats, and canaries.

Free-ranging marine mammals have died of acute toxoplasmosis [92]. Numerous reports exist of *T gondii* infections in marine mammals, including sea otters, dolphins, seals, and whales [92], and toxoplasmosis has been considered a cause of death in sea otters [93–95]; yet, how marine mammals become infected is unknown. Cole and colleagues [93] postulated, based on clinical evidence in sea otters, and Miller and coworkers [95] presented evidence that coastal fresh water surface runoff presented a risk of infection to sea otters; thus, it is possible that *T gondii* oocysts could be washed into the sea via runoff contaminated by cat excrement. *T gondii* infection is widespread in human beings, and the prevalence varies with geography and increase in age. In the United States and the United Kingdom, it is estimated that 16% to 40% of people become infected, whereas in Central American and South America and continental Europe, infection estimates

reach 50% to 80% [61,88,96]. Infections in healthy adults are usually asymptomatic; however, severe disease can occur in immunocompromised individuals and newborns.

Congenital infection may occur after maternal infection during pregnancy. The severity of the disease may depend on the stage of pregnancy at the time of infection. A wide spectrum of clinical disease occurs in congenitally infected children [97,98]. Mild disease may consist of slightly diminished vision only, whereas severely diseased children may have the full tetrad of signs, including retinochoroiditis, hydrocephalus, convulsions, and intracerebral calcification. Of these, hydrocephalus is the least common but most dramatic lesion of toxoplasmosis. This condition is unique to congenitally acquired toxoplasmosis in human beings and has not been reported in other animals.

Postnatally acquired infection may be localized or generalized. Oocyst-transmitted infections may be more severe than tissue cyst-induced infections [84,85]. Lymphadenitis is the most frequently observed clinical form of toxoplasmosis in human beings [61,84]. Although any nodes may be involved, the most frequently involved are the deep cervical nodes. When infected, they are tender and discrete but not painful, and the infections resolve spontaneously in weeks or months. Lymphadenopathy may be associated with fever, malaise, fatigue, muscle pain, and sore throat and headache. Although the condition may be benign, its diagnosis is vital in pregnant women because of the risk to the fetus. Many patients with AIDS and those given immunosuppressive treatments die of acute toxoplasmosis, often involving brain [99].

### Diagnosis

Diagnosis is made by biologic, serologic, or histologic methods or by some combination of these. Clinical signs are nonspecific and insufficiently characteristic for a definite diagnosis, because toxoplasmosis mimics several other infectious diseases.

Numerous serologic procedures are available for the detection of humoral antibodies, including the Sabin-Feldman dye test, indirect hemagglutination assays, IFATs, direct agglutination tests, latex agglutination tests, ELISAs, and the immunoabsorbent agglutination assay test (ISAAGA) [61,98]. The IFAT, ISAAGA, and ELISAs have been modified to detect IgM antibodies, which appear sooner after infection than IgG antibodies and disappear faster than IgG antibodies after recovery.

The finding of antibodies to *T gondii* in one serum sample merely establishes that the host has been infected at some time in the past; thus, it is best to collect two samples from the same individual, the second 2 to 4 weeks after the first. A 4- to 16-fold increase in antibody titers in the second sample indicates an acute infection. A high antibody titer sometimes persists for months after infection. A rise in antibody titers may not be associated

with clinical symptoms, because, as indicated earlier, most infections in human beings are asymptomatic and the fact that titers persist after clinical recovery complicates the interpretation of the results of serologic tests. *T gondii* can be isolated from patients by inoculation into laboratory animals and tissue cultures of secretions, excretions, body fluids, tissues taken by biopsy, and tissues with macroscopic lesions taken after death. Using such specimens, it is possible not only to attempt isolation of *T gondii* but to search for *T gondii* microscopically or for toxoplasmic DNA using the PCR for processing. It is advisable not to rely entirely on this test for making vital decisions involving the fetus.

As just noted, diagnosis can be made by finding *T gondii* in host tissue removed by biopsy or at necropsy. A rapid diagnosis may be made by microscopic examination of impression smears of lesions. After drying for 10 to 30 minutes, the smears are fixed in methyl alcohol and stained with a Romanowsky stain, with a Giemsa stain being satisfactory. Well-preserved *T gondii* are crescent shaped. In sections, the tachyzoites usually appear round to oval. Electron microscopy can aid in diagnosis. *T gondii* tachyzoites are always located in vacuoles; they have few (usually four) rhoptries and often have a honeycomb structure. Tissue cysts are usually spherical and lack septa, and the cyst wall stains with silver stains. The bradyzoites are strongly periodic acid–Schiff (PAS)-positive [61]. The immunohistochemical staining of parasites with fluorescent or other types of labeled *T gondii* antiserum can aid in diagnosis.

### Chemotherapy

Sulfadiazine and pyrimethamine are widely used for therapy of toxoplasmosis [61,100]. 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 do not usually eradicate infection. These drugs seem to have little effect on subclinical infections, but the growth of tissue cysts in mice has been restrained with sulfonamides. Sulfa compounds are excreted within a few hours of administration; thus, treatment has to be administered in daily divided doses. Doses vary with age and the species of the host [100,101]. Spiramycin, clindamycin, atovaquone, azithromycin, roxithromycin, clarithromycin, dapsone, and several other less commonly used drugs are available for treatment of toxoplasmosis. Clindamycin is absorbed quickly and diffuses well into the CNS; therefore, it has been used as an alternative to sulfadiazine [101].

### *Prevention and control*

To prevent infection of human beings by *T gondii*, people handling meat should wash their hands thoroughly with soap and water before going to other tasks [61,102]. All cutting boards, sink tops, knives, and other materials contacting uncooked meat should also be washed with soap and water. Washing is effective because the stages of *T gondii* in meat are killed by contact with soap and water [61]. *T gondii* in meat is killed by exposure to extreme cold or heat. Tissue cysts in meat are killed by heating to an internal temperature of 67°C [103] or by cooling to -13°C [104]. *T gondii* in tissue cysts or oocysts is killed by exposure to 0.5 kilorads of  $\gamma$ -irradiation [105,106]. Meat of any animal should be cooked to a minimum of 67°C before consumption, and tasting meat while cooking or while seasoning should be avoided. High-pressure treatment can kill tissue cysts in meat and oocysts on fruits and vegetables [107,108]. Salt additives to tenderize the meat can reduce inactivate tissue cysts in meat [81,109].

Pregnant women should avoid contact with cats, cat litter, soil, and raw meat. Pet cats should be fed only dry, canned, or cooked food, and the cat litter box should be emptied daily. Gloves should be worn while gardening, and vegetables should be washed thoroughly before eating because they may have been contaminated with cat feces. People should avoid drinking unfiltered water from lakes, ponds, and rivers. Access to water reservoirs by cats should be prevented.

### *Vaccination*

There is no commercial vaccine to prevent *T gondii* infection in cats and human beings. One vaccine that contains a strain (S48) of tachyzoites that does not persist in the tissues of sheep is available in Europe and New Zealand, where it is used to reduce fetal losses attributable to toxoplasmosis [110]. Ewes vaccinated with the S48 strain vaccine retain immunity for at least 18 months [110].

## **Sarcocystosis**

### *Etiologic agent*

Unlike *Neospora* and *Toxoplasma*, the genus *Sarcocystis* has more than 100 species that infect mammals, birds, marsupials, and poikilothermic animals. *Sarcocystis* has an obligatory prey-predator (two-host) life cycle (Fig. 3). Asexual stages develop only in the intermediate host, which, in nature, is often a prey animal, and sexual stages develop only in the definitive host, which is carnivorous. There are different intermediate and definitive hosts for each species of *Sarcocystis*; for example, there are three named species of *Sarcocystis* in cattle: *Sarcocystis cruzi*, *Sarcocystis hirsuta*, and *Sarcocystis hominis* [111], with the definitive hosts for these species being Canidae,

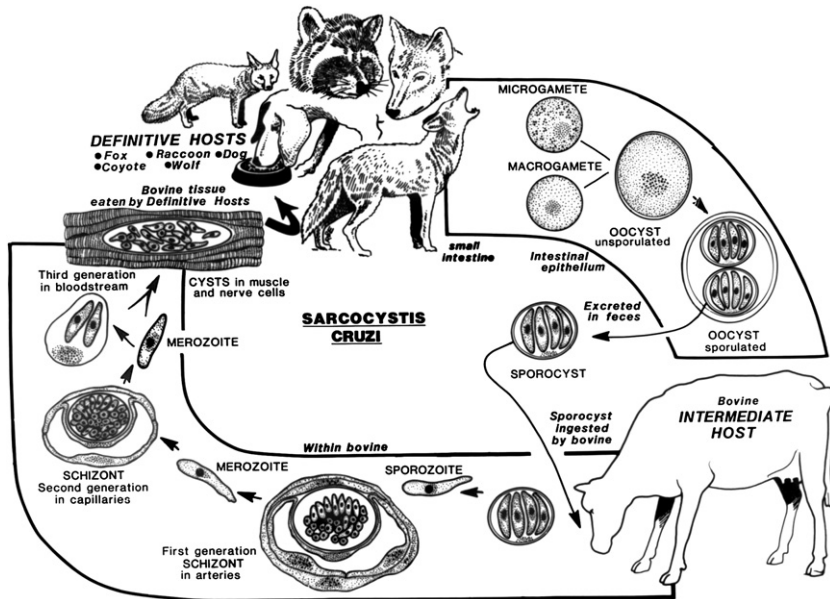


Fig. 3. Life cycle of *Sarcocystis cruzi*. (From Dubey JP, Fayer R. Bovine sarcocystosis. *Comp Contin Edu Pract Vet* 1986;8:130-42.)

Felidae, and primates, respectively. Species of *Sarcocystis* 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 life cycle and structure, *S. cruzi* serves as the example because its complete life cycle is known.

The intermediate host becomes infected by ingesting sporocysts in food or water. Sporozoites excyst from sporocysts in the small intestine, and first-generation schizonts are formed in endothelial cells of arteries 7 to 15 days after inoculation. Second-generation schizonts occur 19 to 46 days after inoculation, predominantly in capillaries virtually throughout the body. Merozoites are found in mononuclear blood cells 24 to 46 days after inoculation.

The schizonts divide by endopolygony, wherein the nucleus becomes lobulated and divides into several nuclei. Merozoites form at the periphery of the schizont. First- and second-generation schizonts are located within the host cytoplasm and are not surrounded by a parasitophorous vacuole. *Sarcocystis* merozoites have the same organelles as do other coccidians, but there are no rhoptries. Rhoptries, however, are present in *Sarcocystis* bradyzoites [111].

Merozoites liberated from the terminal vascular generation of the developing parasite initiate sarcocyst formation. These merozoites penetrate appropriate host cells. The intracellular merozoite, which is surrounded by



a parasitophorous vacuole (PV), becomes round to ovoid (metrocyte) and undergoes repeated division, producing many metrocytes that eventually produce banana-shaped zoites called bradyzoites (also called cystozoites). Some mature sarcocysts may contain some peripherally arranged metrocytes in addition to zoites. Eventually, the sarcocyst is filled with bradyzoites, the stage infective for the predator definitive host. Sarcocysts generally become infectious approximately 75 days after infection, but there is considerable variation among species of *Sarcocystis*. Immature sarcocysts containing only metrocytes and schizonts 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) gamonts. After fertilization of a macrogamete by a microgamete, a wall develops around the zygote and an oocyst is formed. The entire process of gametogony and fertilization can be completed within 24 hours.

Oocysts of *Sarcocystis* species sporulate in the lamina propria. Sporulated oocysts are thin walled ( $< 1 \mu\text{m}$ ). 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 for most *Sarcocystis* species, oocysts are first shed in feces 7 to 14 days after ingesting sarcocysts.

The number of generations of schizogony and the type of host cell in which schizogony may occur vary with each species of *Sarcocystis*, but trends are apparent. For example, all species of *Sarcocystis* of large domestic animals (sheep, goats, cattle, and pigs) form first- and second-generation schizonts in the vascular endothelium, whereas only a single precystic generation of schizogony has been found in *Sarcocystis* species of small mammals (mice and deer mice), and this is generally in hepatocytes [111].

Sarcocysts, which are always located within a PV 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 differ among species of *Sarcocystis* and within each species as the sarcocyst matures [111]. Histologically, the sarcocyst wall may be smooth, striated, or hirsute, or it may possess complex branched protrusions. These protrusions are of taxonomic importance. Internally, groups of zoites may be segregated into compartments by septa that originate from the sarcocyst wall, or they may not be compartmentalized.

Not all species of *Sarcocystis* cause disease in their host species [111]. Generally, species using canids as definitive hosts are more pathogenic than those using felids. For example, of the three species in cattle, *S. cruzi*, for which the dog is the definitive host, is the most pathogenic, whereas *S. hirsuta* and *S. hominis*, which undergo sexual development in cats and primates, respectively, are only mildly pathogenic (Table 1). Pathogenicity is manifested in the intermediate host. *Sarcocystis* generally does not cause illness in definitive hosts.

*S. neurona* is an unusual species of the genus that does not follow the life cycle pattern of *S. cruzi* outlined previously [112]. It is also one of the most pathogenic species of the genus. *S. neurona* is the most frequent cause of a fatal disease in horses called equine protozoal encephalomyelitis (EPM) in North America and South America [112]. Horses are considered an aberrant host, because only schizonts are found in their tissues. Unlike *S. cruzi*, *S. neurona* schizonts occur in neural cells rather than in the vascular endothelium, and schizonts may persist in the CNS for months. Its sarcocysts occur in domestic cats, striped skunks, raccoons, sea otters, and armadillos. Opossums (*Didelphis virginianus* and *Didelphis abbreventis*) are its definitive hosts. Only the sexual cycle occurs in the definitive host, and it is confined to the small intestine. Encephalomyelitis associated with *S. neurona* has been reported in horses, ponies, zebras, skunks, raccoons, cats, dogs, lynx, mink and marine mammals [112,113].

*S. canis* is another unusual species of the genus with an unknown life cycle [113]. Its sarcocysts, sexual phase, and definitive hosts are unknown. The schizont is the only stage that is known. *S. canis* has been found associated with fatal hepatitis in sea lions, dogs, black bears, grizzly bears, a horse, and a dolphin. Congenital infection has been documented in dogs.

#### *Clinical sarcocystosis in animals*

*Sarcocystis* is generally nonpathogenic for the definitive host, and some species of *Sarcocystis* are also nonpathogenic for intermediate hosts (see Table 1). Generally, species transmitted by canids are pathogenic, whereas those transmitted by felids are nonpathogenic. *S. cruzi*, *Sarcocystis capracanis*, and *Sarcocystis tenella* are the most pathogenic species for cattle, goats, and sheep, respectively. Clinical signs are generally seen during the second schizogonic cycle in blood vessels (acute phase). Three to 4 weeks after infection with a large dose of sporocysts (50,000 or more), fever, anorexia, anemia, emaciation, and hair loss (particularly on the rump and tail in cattle) develop, and some animals 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 muscle, 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.

#### *Clinical sarcocystosis in human beings*

Human beings serve as the definitive host for *S. hominis* and *Sarcocystis suihominis* and also serve as accidental intermediate hosts for several

Table 1  
*Sarcocystis* species in livestock

Intermediate host	<i>Sarcocystis</i> species	Reference	Sarcocysts		Pathogenicity <sup>a</sup>	Definitive hosts
			Maximum length (mm)	Wall (mm)		
Cattle ( <i>Bos taurus</i> )	<i>S. cruzi</i>	Hasselmann, 1926; Wenyon, 1926	<1	7	++	Dog, coyote, red fox, raccoon, wolf
	<i>S. hirsuta</i>	Moulé, 1888	7	10	+/-	Cat
	<i>S. hominis</i>	Railliet and Lucet, 1891; Dubey, 1976	7	10	+/-	Man, other primates
Sheep ( <i>Ovis aries</i> )	<i>S. tenella</i>	Railliet, 1886; Moulé, 1886	0.7	14	++	Dog, coyote, red fox
	<i>S. arieticanis</i>	Heydorn, 1985	0.9	7	+	Dog
	<i>S. gigantea</i>	Railliet, 1886; Ashford, 1977	10	21	-	Cat
	<i>S. medusiformis</i>	Collins et al, 1979	8	20	-	Cat
Goat ( <i>Capra hircus</i> )	<i>S. capracanis</i>	Fisher, 1979	1	14	++	Dog, coyote, red fox
	<i>S. hircicanis</i>	Heydorn and Unterhözner, 1983	2.5	7	++	Dog
Pigs ( <i>Sus scrofa</i> )	<i>S. moule</i>	Nevu-Nemaire, 1912	7.5	7	?	Cat
	<i>S. miescheriana</i>	Künn, 1865; Labbé, 1899	1.5	10 (?)	+	Dog, raccoon, wolf, red fox, jackal
	<i>S. porcifelis</i>	Dubey, 1976	?	?	?	Cat
	<i>S. sui hominis</i>	Tadros and Laarman, 1976; Heydorn, 1977	1.5	10	+	Humans, primates

Horses ( <i>Equus caballus</i> )	<i>S fayeri</i>	Dubey et al, 1977	1.0	11	+/-	Dog
	<i>S equicanis</i>	Rommel and Geisel, 1975	0.35	?	?	Dog
Water buffalo ( <i>Bubalus bubalis</i> )	<i>S bertrami</i>	Doflein, 1901	12	?	?	Dog
	<i>S levinei</i>	Dissanike and Kan, 1978; Huong et al, 1997	1.1	7 (?)	+	Dog
	<i>S fusiformis</i>	Railliet, 1897; Bernard and Bauche, 1912	3	21	-	Cat
	<i>S dubeyi</i>	Huong and Uggla, 1999	<1 (?)	9	?	?
Camel ( <i>Camelus spp</i> )	<i>S buffalonis</i>	Huong et al, 1997	8	7.7	?	Cat
	<i>S cameli</i>	Mason, 1910	0.38	?	?	Dog
	<i>S sp</i>	Mason, 1910	?	?	?	?
Chickens ( <i>Gallus gallus</i> )	<i>S horvathi</i>	Ratz, 1908	0.98	?	?	?
	<i>S sp</i>	Wenzel, et al, 1982	?	?	?	Dog, cat
	<i>S rileyi</i>	Stiles, 1893; Michin, 1903	12	23	?	Skunk

Abbreviations: ++, very pathogenic; +, pathogenic; mildly pathogenic; -, nonpathogenic; +/-, questionable pathogenicity; ?, unknown or unclassified.

<sup>a</sup> Modified from references [111,114], where a complete bibliography can be found.

unidentified species of *Sarcocystis* [111,114]. Symptoms vary with the species of *Sarcocystis* causing the infection and organ parasitized. Intestinal sarcocystosis is acquired by ingesting uncooked beef containing sarcocysts of *S hominis* or pork containing *S suihominis*. Symptoms include nausea, stomachache, and abdominal pain. Human volunteers developed hypersensitivity-like symptoms, including nausea, vomiting, stomachache, diarrhea, and dyspnea, within 24 hours of ingestion of uncooked pork from naturally or experimentally infected pigs. Sporocysts were shed 11 to 13 days after ingesting the infected pork or beef [114–121]. Sarcocysts have been found in striated muscles of human beings, mostly as incidental findings [122]. Recently, 7 of 15 US military men developed acute illness after an army exercise in rural Malaysia [123]. The illness was characterized by fever, myalgias, bronchospasm, fleeting pruritic rashes, transient lymphadenopathy, and subcutaneous nodules associated with eosinophilia, an elevated erythrocyte sedimentation rate, and elevated levels of muscle creatinine kinase. Sarcocysts of an unidentified *Sarcocystis* species were found in skeletal muscle biopsies of the index case [123].

#### *Eosinophilic myositis*

Eosinophilic myositis (EM) is a specific inflammatory condition of striated muscles, mainly attributable to accumulations of eosinophils [111,124]. It has been found mainly in cattle, occasionally in sheep, and rarely in pigs and horses. 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* spp [111]. Moreover, the high prevalence of *Sarcocystis* spp infection in naturally infected cattle makes it difficult to designate *Sarcocystis* as the cause of EM. Degrating sarcocysts are found in sections of lesions of EM [124].

Condemnation of beef containing lesions of EM or grossly visible sarcocysts (*S hirsuta*) can be a serious economic problem [125,126]. In one study, 974 of 1,622,402 (0.06%) cattle slaughtered in 1965 through 1966 in the United States were condemned because of EM [126]. In another report, 18 bovine carcasses from one slaughter plant in the United States were condemned because of grossly visible *S hirsuta* sarcocysts [125].

#### *Diagnosis*

The antemortem diagnosis of muscular sarcocystosis can only be made by histologic examination of muscle collected by biopsy [111,127,128]. The finding of immature sarcocysts with macrocytes suggests recently acquired infection, and the finding of mature sarcocysts indicates only past infection [111].

An inflammatory response associated with sarcocysts may help to distinguish an active disease process from an incidental finding of sarcocysts.

*Sarcocystis* schizonts have not yet been identified in human beings. Although there are several serologic tests and PCR techniques developed experimentally to distinguish *Sarcocystis* species in animals, none have been applied to cases of sarcocystosis in human beings. Tenter [127] has reviewed in detail pitfalls of serologic and molecular diagnosis of sarcocystosis in animals.

The diagnosis of intestinal sarcocystosis is easily made by fecal examination. As has been mentioned, sporocysts or oocysts of sarcocystis are shed fully sporulated in feces, whereas those of *Isospora belli* are often shed unsporulated. It is not possible to distinguish one species of *Sarcocystis* from another by the examination of sporocysts.

### *Epidemiology and control*

*Sarcocystis* infection is common in many species of animals worldwide [111]. A variety of conditions permit such high prevalence: a host may harbor any of several species of *Sarcocystis*; many definitive hosts are involved in transmission; large numbers of sporocysts may be shed; *Sarcocystis* oocysts and sporocysts develop in the lamina propria and are discharged over a period of many months; oocysts and sporocysts are resistant to freezing and can overwinter on the pasture, or they may be spread by invertebrate transport hosts; there is little or no immunity to reshedding of sporocysts, and each meal of infected meat can thus initiate a new round of sporocyst production; and the fact that *Sarcocystis* oocysts, unlike those of many other species of coccidia, are passed in feces in the infective form frees them from dependence on weather conditions for maturation and infectivity.

Poor hygiene during handling of meat between slaughter and cooking can be a source of *Sarcocystis* infection. In one survey in India, *S. suihominis* oocysts were found in feces of 14 of 20 3- to 12-year-old children [121], indicating that meat was consumed raw at least by some, because *S. suihominis* can be transmitted to human beings only by the consumption of raw pork. In another study, 3- to 5-year-old children from a slum area were found to consume meat scraps virtually raw, and many pigs from that area harbored *S. suihominis* sarcocysts [120]. In European countries, where the frequency of consumption of raw or undercooked meat is relatively high, human beings are likely to have intestinal sarcocystosis. There is no treatment for *Sarcocystis* infection of human beings. On the basis of results in experimental animals, it is probable that sulfonamides and pyrimethamine may be helpful in treating sarcocystosis. There is no vaccine to protect livestock or human beings 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 reduce or eliminate infectious sarcocysts, meat should be frozen if not cooked.

Exposure to heat at 55°C for 20 minutes kills sarcocysts; thus, only limited cooking or heating is required to kill sporocysts [129]. Dead livestock should be buried or incinerated. Dead animals should never be left in the field for vultures and carnivores to eat.

## Summary

In conclusion, much needs to be learned concerning the prevention of *N caninum*, *T gondii*, and *Sarcocystis* spp infections in livestock. Neosporosis is an enigmatic infection of cattle, and inducing immunity to congenital transfer of *N caninum* is a challenge for immunologists, parasitologists, and veterinarians. Further research is needed regarding the pathogenesis of *N caninum* abortion, the life cycle of the parasite in cattle, and sources of infection. Prevention of transmission of *T gondii* in pregnant women is a major concern. Effects of *Sarcocystis* infections in livestock are difficult to evaluate, because nearly all cattle are infected with *S cruzi*.

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