REVIEW ARTICLE

Bovine Neosporosis

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Abstract

Neospora caninum is the protozoan parasite that causes bovine abortion worldwide. *N. caninum* has three infective stages of parasite, i.e. tachyzoites, bradyzoites and oocysts, and a two-host life cycle, including definitive hosts and intermediate. The domestic dogs (*Canis lupus familiaris*), coyotes (*C. latrans*) and the Australian dingoes (*C. lupus dingo*) are known as definitive host whereas cattle and other species such as water buffaloes, pigs, red foxes, white-tailed deer, gerbils and monkeys serve as intermediate hosts of the parasite. Vertical (endogenous transplacental) transmission is the dominating route of *N. caninum* infection. Horizontal or exogenous transmission may occur when animals become infected after birth. Dogs become infected and shed the oocysts in their faeces after consuming tissues, or placenta from cattle infected with *N. caninum*. Abortion is the most common clinical sign in adult cattle and can occur throughout pregnancy. A variety of diagnostic methods can be used for detection of *N. caninum* infection including isolation of viable parasite, histology, polymerase chain reaction and serology. Economic losses from *N. caninum* infection are associated with abortion, increased culling and reduced milk yield, stillbirth and neonatal mortality. Currently, no drug or chemotherapy is available for effective treatment of bovine *N. caninum* infection or to prevent transmission of the parasite from an infected dam to her offspring. Control measures to avoid *N. caninum* infection are removal of infected cows, not breeding replacement heifers from infected cows, removal of aborted foetuses, dead calves, and placentas from infected cows.

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Introduction

Neospora caninum is a cyst-forming intracellular protozoan parasite that belongs to the family Sarcocystidae within the phylum Apicomplexa. It was first identified in Norwegian dogs with encephalomyelitis and myositis in 1984 [1] and later was named in 1988 [2]. However, the retrospective studies have revealed *N. caninum* parasites in dogs as early as 1957 and in a stillborn calf in 1974 [3]. *N. caninum* is closely related to *Toxoplasma gondii,* but the two parasites are distinct in both ultrastructure and antigenicity [4]. *N. caninum* isolates from dogs and aborted bovine foetuses are shown to be the same species [5].

Biology and life cycle of *N. caninum*

Tachyzoites, bradyzoites and oocysts have been identified as the infective stages of the parasite [6, 7]. Tachyzoites and bradyzoites are asexual stages of the parasite. They are found in different cell types and organs, most often in the brain and spinal cord [8]. In pregnant cattle, tachyzoites have been found in the placenta [9]. The tachyzoites can multiply rapidly in the host cell resulting in cell death and necrotic lesions. Bradyzoites usually inhabit tissue cysts in the central nervous system and other neural tissues. Tissue cysts are round to oval in shape, and with a thick cyst wall [8]. Oocysts are the result of sexual reproduction of *N. caninum* and are spherical to subspherical. Non-infective unsporulated oocysts are shed in faeces of a definitive host and sporulate and become infective within three days in the environment [6, 7]. The sporulated oocysts have two sporocysts, each with four sporozoites. *N. caninum* oocysts are morphologically similar to *Hammondia heydorni* found in dog faeces, and *Toxoplasma gondii* and *Hammondia hammondi* found in cat faeces [10, 11].

N. caninum has a two-host life cycle, including intermediate and definitive hosts. The domestic dog (*Canis lupus familiaris*) is both a natural and experimental definitive host for *N. caninum* [6, 7]. In addtion, wild dogs, i.e. the coyotes (*C. latrans*) and the Australian dingoes (*C. lupus dingo*), have also been experimentally demonstrated as definitive hosts of *N. caninum* [12, 13]. Recently, grey wolves (*C. lupus*) were confirmed to be natural definitive hosts *of N. caninum* by shedding of viable *N. caninum* oocysts in their feces [14]. Dogs become infected and shed oocysts with their faeces after ingesting infected tissues of intermediate hosts [6, 7, 12]. In additions, it was shown that a puppy can produce and shed more oocysts than an adult dog [15]. Cattle and other animal species such as water buffaloes, pigs, red foxes, white-tailed deer, gerbils and monkeys can serve as intermediate hosts of the parasite [16]. The intermediate hosts become infected after ingestion of oocysts-contaminated feed and water, or by eating tissues of infected animals [12, 17, 18]. There are still uncertainties concerning the survival of oocysts in the environment. There is no evidence that avian species are natural hosts for *N. caninum* [19].

Prevalence

Serologic prevalences of *N. caninum* antibodies in different species of mammals including dogs, dairy cattle, beef cattle, other domestic animals, wildlife and zoo animals, and humans have been reported from several countries over the world [16]. These results provide evidence that many species of mammals have been exposed to this parasite. However, these results are not comparable because of different serological methods and different cut-off values used. Neosporosis is now considered as the most frequently diagnosed cause of abortion in cattle worldwide [20]. It seems that the estimated serological prevalences of *N. caninum* infection in cattle are considerable differences among countries, within countries, between regions, animal species and study designs [16, 21, 22]. In an European study, seroprevalences were compared in dairy and beef cattle from Germany, The Netherlands, Spain, and Sweden by use of randomized samples and enzyme-linked immunosorbent assays (ELISAs) that had been previously standardized among laboratories [23, 24] . In this study, the seroprevalence in cattle in Sweden was much lower than in neighboring countries and prevalences in beef cattle were lower than in dairy cattle. Between 16% and 83% of dairy herds were confirmed to have *N. caninum* infected animals [16, 23]. However, the *N. caninum* seropositivity of cattle in the infected herds varies considerably and, in some dairy herds, up to 87% of cattle are seropositive [16, 25-27].

Mode of transmission

Transplacental (vertical) and horizontal (postnatal) transmission are known as the route of *N. caninum*-infection. Vertical transmission, i.e. endogenous transplacental, is the most frequent route of *N. caninum* infection in cattle [27-30], but little is known of the mechanism for this. Evidence for this efficient transplacental transmission comes from several sources: familial, comparison of antibody status in cows and their offspring, infection status of offspring, and experimental. Results obtained from studies with dam and progeny indicate that up to 95% of calves were born infected but asymptomatic [16]. Repeated vertical transmission can occur in subsequent pregnancies and a congenitally infected heifer can herself give birth to congenitally infected calves [28, 30, 31]. The rate of endogenous transplacental infection may decrease in subsequent pregnancies, indicating immune responses [32, 33]. Endogenous transplacental transmission occurs in a persistently infected dam after reactivation (recrudescence) of the infection during pregnancy. By congenital transmission, *N. caninum* infection can keep spreading in a herd for several years.

Mathematical models of *N. caninum* infections within dairy herds indicate that vertical transmission alone cannot sustain infection within herds [34]. Transmission by endogenous transplacental infection is below 100% that would lead to a continuous decrease in infection prevalence in the infected herds. Horizontal or exogenous transmission, i.e. that animals may become infected after birth, is essentially required to maintain infection within a herd [34]. Postnatal acquisition has experimentally been verified to occur showing that cattle may be infected when they consume oocysts [12]shed by definitive hosts such as dogs and coyotes [7, 13, 14, 35]. Dogs become infected and shed the oocysts in faeces within two weeks after consuming tissues [36], or placenta [37]from cattle infected with *N. caninum*. Newborn calves may become orally infected after ingestion of colostrum or milk replacer contaminated with tachyzoites [38, 39], but the finding of parasites in colostrum or milk from naturally infected cows has never been reported. Cattle can probably be infected by ingesting tissues that contain tachyzoites or tissue cysts [9].

Clinical findings

Abortion is the only clinical sign in adult cattle that are infected with *N. caninum*. Abortion can occur throughout pregnancy, but is most common between five to seven months of gestation [32, 40]. *N. caninum*-associated abortion may take place throughout the year and may have an epidemic or endemic patterns. Epidemic abortion means that a large number of the pregnant cows in a herd experienced an abortion temporary and if 15% of the cows at risk abort within 4 weeks, 12.5% of the cows abort within 8 weeks, and 10% of the cows abort within 6 weeks [41, 42]. In contrast, the term endemic abortion refers to the situation when abortions occur throughout the year, and during several years. Possibly, these two patterns of *N. caninum*-associated abortion are related to routes by which *N. caninum* infections can cause abortion [43]. Epidemic abortions are thought to be due to naive dams become infected with *N. caninum* after ingestion of feed or water contaminated with *N. caninum*-oocysts [17, 44]. When pregnant dams have been exposed to contamination with oocysts virtually at one time (point source exposure), exogenous transplacental infection and the abortions may occur within a short period of time. The finding of low-avidity immunoglobulin G (IgG) responses suggests an acute infection in herds with epidemic abortion [17, 41, 45, 46]. Recrudescence of a latent infection in the dam during gestation may cause abortion [47-49]. Latent infection in dams may have been acquired transplacentally or postnatally [50, 51]. The mechanism of reactivation of latent *N. caninum* infection is not known. Reactivation may be induced by immune suppression after ingestion of toxic feeds or other concurrent infections such as bovine viral diarrhea virus [42, 52]. Recently, it was shown that progesterone supplementation during midgestation increases the risk of abortion in *Neospora*-infected dairy cows with high antibody titers [53].

Experimental studies have shown that *N. caninum* infection produces the detrimental effects on reproductive performance of cattle. The infections during early pregnancy are more likely to cause foetal death and resorption rather than abortion [54, 55]. Some studies have found that seropositive heifers or cows required more inseminations per confirmed pregnancy than their seronegative herd mates [15, 56], indicating that early foetal loss has occurred. However, a Spanish study suggests that *N. caninum* infection does not appear to affect the foetus survival during early gestation [57]. Infection at mid-gestation may result in abortion or the birth of a persistently infected calf [55, 58]. However, with infection in late pregnancy, cows usually deliver normally and most calves are clinically healthy but congenially infected

[55, 58, 59]. Seropositive cows do not necessarily abort although they may have up to 19 times increased risk of abortion compared with seronegative ones [31, 52, 56, 57, 60]. Seropositive primiparous cows had 7.4 times higher risk of abortion and this risk decreased in subsequent pregnancies [60]. Repeated abortions can occur in the same cows, but its frequency is not known [31, 50, 60].

Most calves born to *N. caninum*-infected heifers or cows are clinically normal, but congenitally infected with the parasite [61]. However, congenitally infected calves carried to term may also be stillborn, or alive but underweight, unable to rise and exhibit variable neurological deficits. Neurological signs consist of ataxia, with decreased patella reflex and a slight loss of conscious proprioception in either hind limbs, or all limbs, and exophthalmia or an asymmetrical appearance of eyes [10]. It has also been reported that some congenitally infected calves with good health at birth become progressively weaker within a few weeks after birth.

Diagnosis

Accurate diagnosis of *N. caninum*-infected animals is important for a better understanding of the epidemiology of neosporosis. Isolation of the viable parasite is the most definitive evidence of *N. caninum* infection, but attempts to retrieve viable isolate may be unsuccessful if the parasite die with the host [20].

Histopathological examinations and immunohistochemistry (IHC) are frequently used methods for demonstration of *N. caninum* infection in bovine foetal tissues. Presence of the parasite in the tissue is a definitive diagnosis of infection but the sparse distribution of parasites makes detection difficult. Brain, liver, heart, and placenta are essentially required specimens for histology and diagnostic rates are higher if multiple tissues are examined [8, 20]. IHC methods utilizing specific antibodies to *N. caninum* are used to identify the parasites with highly specific, but is laborious and has a low sensitivity [62].

Polymerase chain reaction (PCR) has been developed to amplify *N. caninum* DNA in different tissues from aborted bovine foetuses such as brain, spinal cord, heart, placentas, and in amniotic fluid of infected cattle[8, 62, 63]. Moreover, PCR has been applied to detect *N. caninum* oocysts in faeces of definitive hosts i.e. dogs and dingoes [12, 13]. PCR assays are highly sensitive and specific, and less affected by autolysis than are histopathology.

Presence of specific antibodies to *N. caninum* indicates that an individual or a herd has been exposed to the parasite. A variety of antibody assays, including the indirect fluorescent antibody test (IFAT), immunoblot, direct agglutination test, and various ELISAs, have been used for detection of specific antibodies to *N. caninum*. Different samples including serum, milk, foetal blood, plural and peritoneal fluids are used for such methods [64, 65]. Serum is the most commonly used materials for demonstration of antibodies to *N. caninum* in adult animals. However, *N. caninum* specific antibodies can also be found in vaginal secretion and saliva [66], and milk [26, 41, 67-70].

Both the age and the stage of the autolysis of foetus may affect the sensitivity of the antibody assays, e.g. IFAT and ELISA [40, 62], when is used. Analysis of fluids collected from bovine foetus younger than six months old may cause false negative results because the immune system is not fully developed [40]. Also, severe autolysis can initiate the degradation of the immunoglobulins of the foetuses. Serological test might be used on newborn calves to determine whether they are congenitally infected. Comparison of seroprevalences in dams and their offspring is useful to estimate the rate of transplacental transmission of *N. caninum* [16]. However, it is important to collect a blood sample from the calf before feeding colostrum because colostral antibodies from the dam may persist in the calf for several months [31]. Serological test is an invaluable tool to determine whether an aborting cow or a herd experiencing an abortion problem has been exposed to *N. caninum* [18, 31, 41]. Careful consideration should always be given when interpreting the serologic results [20, 50]. Most naturally infected cows are seropositive at the time of abortion [47, 48]. However, as the antibody levels fluctuate during pregnancy [48], an infected cow may, sometimes, be seronegative [71].

IgG avidity ELISAs have also been developed to discriminate between chronic and acute infections caused by *N. caninum* [17, 41, 45, 46]. The principle of the IgG avidity test is that the binding strength of *N. caninum* antibodies (affinity) increases in chronically infected animals. A low avidity value thus indicates a recent infection.

Individual milk samples of lactating cows can be used as alternative materials for either screening or diagnosis of *N. caninum* infection [26, 67-70, 72]. The use of milk samples is simpler and non-invasive for animals than blood sampling but also more cost-effective since the farmers themselves can collect the milk sample and deliver it to the laboratory. Because there are still uncertainties about whether subclinical mastitis affects the test result, composite milk is recommended for detection of *N. caninum* antibodies at individual lactating cows. However, studies have reported that a large variation of antibody levels both in milk and serum during lactation in the antibody-positive cows [48, 68]. Stage of lactation may affect the *N. caninum* antibody levels in individual milk. Thus a decrease antibody level in milk as lactation proceeds can be expected. The significant decrease antibody level in milk some months after calving can also be a result of the dilution effect of the increased milk production [73, 74].

The antibody assay can also be applicable to determine *N. caninum* specific antibodies in bulk milk from dairy herds [21, 26, 69, 70, 72]. Bulk milk is a pooled sample and represents all lactating cows. Many factors can therefore influence the performance of the test results e.g. the proportion of infected cows, milk yield and antibody levels of lactating cows [26] and lactation stage [68]. Bulk milk antibody testing can be used to identify *N. caninum*-infected herds. However, a single seropositive cow could considerably influence the bulk milk test result when the herd is small [25, 26]. The use of single bulk milk testing result can lead to misclassification of the herd antibody-status because of the bulk milk result excludes information about non-lactating cattle (e.g. dry cows, heifers and calves) and lactating cows that do not contribute to the bulk milk. Therefore, a single bulk milk test result should be interpreted with caution, especially in small herds where only a few cows contribute to the bulk milk and some seropositive cows may be dried-off. Repeated bulk milk antibody testing could increase the predictability of a herd's true antibody status and the performance of the used test is improved when herd status is interpreted in combination [69]. Bulk milk antibody testing at regular intervals provides better information about herd *N. caninum* infection status than a single test [26, 69]. Moreover, bulk milk antibody testing is a suitable tool for farmers to monitor their herd status over time.

Immunoblot is also considered as a reliable method with a high sensitivity and specificity for antigen/antibody detection of *N.caninum* infection. Such method is widely used to evaluate serological assays performances [75].

Economic impact

Neosporosis is recognized to be a cause of substantial economic losses to both the dairy and the beef industry, but there are no conclusive data on its economic impact. Abortion appears to be the main cause of economic loss both direct and indirect costs [76-78]. In Europe, direct economic losses due to *N. caninum* infection have been estimated to be $11 million per year in Swiss dairy cattle [79] and $2572 annually in Dutch dairy herds that experienced *N. caninum*-associated abortion epidemic [80]. Moreover, replacement cost for the infected herds can increase because heifers that experienced an abortion are more likely to be culled than their non-aborting herd mates [52, 60, 61, 78]. Effects of *N. caninum* infection on milk production are mentioned in the literature, with varying, i.e. positive [52, 56], and negative [81-83] impacts recorded. Neosporosis can cause of other economic losses e.g. repeat breeding, loss of foetuses, stillbirth or birth of weak calves [78, 84].

Prevention and control

Different options for control *N. caninum* infection in cattle have been modeled although the most effective option might not necessarily be optimal from an economic aspect [85]. Test and cull has been used for eradication of the infection from a herd [56]. A major route of *N. caninum* infection in cattle herds is from a dam to her foetus. A basic approach to reduce the infection rate is therefore to remove infected cows. The rationale for culling is that most infected cows can be expected to give birth to an infected calf, and that congenital infection appears to be life-long [27, 28, 61].

Not breeding replacement heifers from infected cows is another strategy to control *N. caninum* infection in herds with a high rate of vertical transmission. This reduces the number of infected animals in the herd by blocking transplacental infection. In addition, both purchase and selective retention of seronegative heifer calves can reduce the prevalence of seropositive animals in the herds [29, 34, 56]. Experimental data have indicated that *N. caninum* is not transmitted by embryo transfer (E.T.) from seropositive donors to seronegative cows. Thus, E.T. can be an alternative method to prevent congenital infection if only seronegative recipients are used [86-88].

Dogs play an important role for *N. caninum* infection in cattle. Specific measures would include removal of aborted foetuses, dead calves, and placentas from infected cows so that dogs cannot eat them. Exposure of cows to faeces of definitive hosts could be reduced by minimizing the number of dogs (and other suspected intermediate hosts, e.g. other canids) in the herd, covering feeds and commodities to prevent contamination by dog faeces [17, 18, 44, 86, 89].

Vaccination [90] and chemotherapy [91] are other control options for neosporosis. Vaccination with a killed tachyzoites formulation is estimated to be only 50% efficacious success rate in cattle (Romero et al., 2004). However, it has not yet been shown that this vaccine is efficient in preventing vertical infection or abortion [90, 92, 93]. No drug or chemotherapy is available for effective treatment of bovine *N. caninum* infection or to prevent transmission of the parasite from an infected dam to her offspring. An epidemiological model suggests that the ‘‘do nothing’’ option is the optimal economic decision to the farmer if a within-herd prevalence is up to 18%. However, vaccination has better economic benefits when the within-herd prevalence of *N. caninum*-infection exceeds 18%.

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