NEOSPORA CANINUM AND NEOSPOROSIS IN ANIMALS – A REVIEW

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Summary


The cyst-forming protozoan Neospora caninum, discovered in 1984 in dogs with encephalitis and myositis, is described as a separate species. The biological cycle of N. caninum involves two hosts: definitive and intermediate. Known definitive hosts are dogs and coyotes. As intermediate hosts, cows, sheep, goats, horses and deers are reported. The dog could be both intermediate and definitive host. The life cycle of N. caninum is characterized by 3 infection stages tachyzoites, tissue cysts and oocysts. The transmission routes of N. caninum are two: horizontal or oral and vertical or transplacental. The definitive hosts shed oocysts that are a potential source of horizontal transmission via infected food and water. At the same time, the vertical transmission from infected dams to their offspring, is also one of the natural routes of infections in cows. Transplacental infection could recur in the same animals. In cows, abortions are the main clinical signs. The cows at various ages could abort from the 3rd months of gestation onward, but abortions by the 5th – 6th month are predominant. Two categories of abortions are distinguished: epidemic, when a higher percentage of cows abort within few weeks and endemic, when abortions occur within months or years. The serological analysis of sera from cows with abortions is indicator of a N. caninum infection. The final diagnosis is made through histological and immunohistological analysis of specimens from foetuses or by detecting N. caninum DNA by PCR. The epidemiology of infection and related abortions is still inadequately studied. The entity of risk factors, influencing the infection and related abortions at the farm level is still incompletely identified. The annual economical losses due to decreased production and losses from aborted foetuses amount to millions of dollars. By reducing the risk of faecal contamination of food and water and destroying aborted foetuses and placentas, the possibility for infection of potential hosts of N. caninum could be limited. In order to prevent the vertical transmission, serological investigations in animals and creation of reproductive programmes in farms according to the seroprevalence are recommended.

The present review attempts to summarize both the known information and our views about the life cycle of the parasite, the pathogenesis and clinical signs of the disease, the mechanisms of transmission, the diagnostic methods and the means of infection control.

Key words: abortions, cattle, dogs, goats, horses, life cycle, Neospora caninum, parasitic diseases, wild animals

INTRODUCTION

Neospora caninum is a recently discovered protozoan that was wrongly identified as Toxoplasma gondii up to 1988. A disease resembling neosporosis was reported for the first time by Bjerkas et al. (1984). The authors identified a cyst-forming protozoan in newborn puppies with nervous signs in Norway. In sera
from these dogs, no antibodies against *T. gondii* have been detected. Dubey et al. (1988a) found similar parasites in 10 dogs in the USA. Having differentiated these parasites from *T. gondii*, the authors referred them to a new genus – *Neospora*, while the species was determined as *Neospora caninum*.

*N. caninum* is highly pathogenic for cows and dogs and could also induce a disease in sheep, goats, horses and deers. The infection with *N. caninum* is one of the main causes of abortions in cows in many countries over the world. The economical losses caused by abortions, reduced productivity and reproductive pathology are estimated to millions of dollars.

The literature reviews of Dubey & Lindsay (1996), Dubey (1999, 2003a, 2003b) and Toolan (2003) provide information about the biology, epidemiology, pathogenesis, clinical manifestation, diagnosis and prophylaxis of neosporosis in animals, updated with the newest scientific facts. So far, not all hosts of the protozoan as well as the routes of transmission, the risk factors for the infection, the methods of prevention and control are determined.

The incomplete information that is available so far and the necessity of complex knowledge on the problem provoked the performance of a detailed assessment and analysis of existing literature data and the composition of the present literature review.

**GENERAL FEATURES OF N. CANINUM INFECTION**

**Biological cycle**

*N. caninum* is an Apicomplexa coccidian protozoan. Its biological cycle involves the participation of two hosts. Dogs (McAllister et al., 1998) and recently coyotes (Gondim et al., 2004) are described as definitive hosts. Apart definitive, the dog could be an intermediate host too (McAllister et al., 1998; Lindsay et al., 1999a, 1999b; Basso et al., 2001a; Dubey et al., 2002). As intermediate hosts of *N. caninum* are shown also cows (Dubey et al., 1990), goats (Dubey et al., 1992; Barr et al., 1992), horses (Daft et al., 1997) and deers (Dubey et al., 1996b). Antibodies against *N. caninum* are also detected in sera from naturally infected buffaloes, coyotes, red foxes and camels and it is suggested that these species are also natural intermediate hosts (Lindsay et al., 1996; Buxton et al., 1997; Simpson et al., 1997; Dubey et al., 1998). According to Bartles et al. (1999) and Ould-Amrouche et al. (1999) birds could also be reservoir hosts, but this hypothesis needs further confirmation.

The life cycle of *N. caninum* comprises three infection stages: tachyzoites, tissue cysts containing bradyzoites and oocysts. Tachyzoites and tissue cysts are stages, detected in intermediate hosts. Both forms are intracellular and are localized in host cell’ cytoplasm, with or without parasitoform vacuole. The size of tachyzoites is about 6 × 2 µm. In infected intermediate hosts they are found in nervous cells, macrophages, fibroblasts, vascular endothelial cells, myocytes, renal tubular epithelial cells and hepatocytes (Dubey et al., 1988a; Dubey & Lindsay, 1989; Dubey et al., 2002).

Tissue cysts are with an oval or round shape and size up to 107 µm. They are detected in the brain, the spinal cord and the retina of intermediate hosts. The bradyzoites enclosed into the cyst are 7–8 × 2 µm in size (Dubey et al., 1988a; Dubey & Lindsay, 1996). Oocysts with dimen-
sions 11.7 × 11.3 µm are shed with the faeces of definitive hosts. They sporulate in the environment and become infective for intermediate hosts (Lindsay et al., 1999a; Lindsay et al., 2001; Slapeta et al., 2002).

Dogs, as definitive hosts, are infected through eating of foetal membranes, placentae, organs of aborted foetuses containing tachyzoites or tissue cysts (McAllister et al., 1998; Lindsay et al., 1999a). A sexual reproduction takes places in canine intestines and consequently, non-sporulated oocysts are shed for 5 to 17 days after the infection with tissue cysts. The oocysts’ size is 11.7 × 11.3 µm and they sporulate in the environment for 3 days. Sporulated, infective oocysts contain 2 sporocysts with 4 sporozoites in each (Lindsay et al., 1999a; Lindsay et al., 2001; Slapeta et al., 2002). Despite the opinion of cited authors that dogs are shedding a small number of oocysts, Gondim et al. (2002) reported recently that dogs, fed tissues from experimentally infected cows, shed more than 100 000 N. caninum oocysts. Yet, little is known about the frequency of oocyst shedding, the time of their survival in the environment or whether other canines are their definitive hosts.

Intermediate hosts could be infected via food and water contaminated with sporulated N. caninum oocysts. Sporozoites are freed into the intestines, penetrate the host cells and then, became transformed into tachyzoites. The latter are rapidly replicated by endodyogeny in host cells and after destroying them, penetrate into new, intact cells (Dubey, 1999; Innes et al., 2001). In nervous cells, tachyzoites could be transformed into bradyzoites (slowly replicating via endodyogeny), when a strong immune response against the protozoan is present. Around the bradyzoites, tissue cysts are formed and within, they remain in a latent state. In immunosuppressive states of the intermediate host (McAllister et al., 1998; Dubey, 1999) bradyzoites could be reactivated.

Tachyzoites could be transmitted vertically from the mother to the foetus via the placenta in case of primary infection with oocysts or during reactivation of tissue cysts. The infection of the embryo could result in abortion, neurological affection of offspring or congenital infection without clinical manifestation. A repeated transplacental infection in the same animals is possible (Dubey & Lindsay, 1996; Davison et al., 1999).

A transplacental infection was experimentally reproduced in cows, sheep, mice, dogs, cats and swine (Dubey et al., 1992; Barr et al., 1993; McAllister et al., 1996; Buxton et al., 1997, 1998; Jensen et al., 1998).

Clinical signs

The commonest clinical signs in dogs younger than 6 months are paresis and paralysis of hindlimbs. Infected dogs could also exhibit difficulties in swallowing, myocarditis, dermatitis and pneumonia (Hay et al., 1990; Odin & Dubey, 1993; Dubey et al., 1995; Greig et al., 1995; Ruehlmann et al., 1995).

Abortions are the first clinical sign in intermediate hosts. The liveborn congenitally infected animals often do not manifest clinical signs of disease. In other cases they could be with a lower body weight at birth, no weight gain, with contracted or strongly extended limbs and neurological signs including ataxia, decreased patellar reflex and lack of orientation (Dubey, 1999).
Pathoanatomical changes

*Neospora caninum* causes cellular death accompanied by visible necrotic lesions in several days due to active replication of tachyzoites. This could result in severe nervous and muscular disease because of nervous cell destruction, including brain and spinal cord cells and due to the effect upon the conductivity of infected cells (Mayhew *et al.*, 1991; Dubey & Lahunta, 1993).

Tissue cysts are often surrounded with a cellular reaction zone. Although the duration of tissue cysts’ persistence is not known, Lindsay *et al.* (1992) observed that they remain vital in the tissues of experimentally infected mice for an year. The formation of granulomas around degenerated tissue cysts and bradyzoites was probably due to formation of inflammation foci consequently to host response to their destruction (Dubey *et al.*, 1992, 1996b).

In 85% of cases, the lesions of aborted foetuses are localized in the central nervous system, although the heart, skeletal muscles, liver and kidneys could also be affected (Barr *et al.*, 1990; Anderson *et al.*, 1991; Wouda *et al.*, 1997).

Epidemiology

After detecting that the definitive hosts of *N. caninum* shed oocysts with faeces, the horizontal route of transmission was discovered by McAllister *et al.* (1998). Regardless of the successful experimental infections of intermediate hosts with oocysts, the transmission mode of infection in natural conditions is still not clear.

In intermediate hosts, the vertical route of transmission from infected cows to offspring is the best studied (Pare *et al.*, 1996; Anderson *et al.*, 1997). Experimentally, the colostrum has also been shown as carrier of infection (Uggla *et al.*, 1998).

In dogs the efficacy of vertical transmission is very variable although in some publications, a transmission from a bitch to her puppies is reported (Dubey *et al.*, 1988b, Dubey & Lindsay, 1989).

By now, a comparative evaluation about the role of horizontal and vertical routes of transmission was not performed.

**Diagnosis**

The oocysts of *N. caninum* in definitive hosts are detected by investigations of faeces using flotation methods (McAllister *et al.*, 1998; Lindsay *et al.*, 1999a). Morphologically, they are identical to oocysts of *Toxoplasma gondii* and *Hammondia hammondii* in feline faeces and *Hammondia heydorni* in canine faeces (Schares *et al.*, 2001a; Dubey *et al.*, 2002). As the microscopy is not enough for differentiation between *N. caninum* from *H. heydorni* oocysts, Hill *et al.* (2001) and Slapeta *et al.* (2002) have developed genetic methods for differentiation of species.

Serologically, it could not be said whether the dog sheds oocysts because the presence of titres does not correlate with oocyst shedding (McAllister *et al.*, 1998).

The dogs as intermediate hosts in whose tissues unsexual replication occurs, manifest clinical signs such as hindlimb hyperextension resulting in progressive spastic paralysis, only in rare cases. Regardless of manifested signs, such dogs do not shed oocysts (Dubey *et al.*, 1988a; Scheahan *et al.*, 1993; Barber & Trees, 1996).

The detection of antibodies against *N. caninum* in intermediate hosts is performed by several serological tests: indirect fluorescence assay (IFA), immunosorbent analysis (ELISA), direct agglutination (DA) and Western blot analysis.
The positive findings of applied serological tests indicate only that the animal has been infected with *N. caninum*, but do not provide a definitive diagnosis in case of abortions. The analysis of tissues from aborted foetuses for presence of specific lesions, tissue cysts and tachyzoites is necessary for confirmation of diagnosis. Specimens of choice are those from the brain although most often, tissue cysts and tachyzoites could be detected in lungs, kidneys and skeletal muscles too. Specific lesions are determined by histological examination of tissues but the identification of the causative agent is performed via immunohistochemical analysis and polymerase chain reaction (PCR) (Lindsay & Dubey, 1989; Dubey & Lindsay, 1996; Jenkins et al., 1997; Cole et al., 1993; Müller et al., 1996; Gottstein et al., 1998).

**Prevention and control**

Both the prevention and control are directed towards reduction of postnatal and congenital infection with *N. caninum*. The restriction of postnatal infection is focused on decreasing the risk of infection with oocysts, shed with the faeces of definitive hosts. For this purpose, reduction of populations of farmers’ dogs and limitation of their access to food and water sources of intermediate hosts, to carcasses of aborted foetuses or dead animal are recommended (McAllister et al., 1998; Toolan, 2003).

As the congenital transmission is most commonly encountered in cows, the prevention measures are related to determination of seroprevalence in the respective farm and histopathological study of aborted foetuses (Pare et al., 1996; Davison et al., 1999). In highly infected farms, the results of serological studies are used in the elaboration of reproductive schedules.

At present, there are no effective means for treatment of neosporosis. There is a vaccine produced by Intervet and licensed in the USA but the results of its application in practice are still under observation.

**NEOSPOROSIS IN DOGS**

**Biological cycle**

The experimental infection in dogs and subsequent oocyst shedding is reproduced after feeding with brain (McAllister et al., 1998; Lindsay et al., 1999a; Gondim et al., 2004; Rodrigues et al., 2004), various tissues or carcasses, including brain or spinal cord (Scharces et al., 2001a; Gondim et al., 2002), heart and skeletal muscles (Scharces et al., 2001a) of intermediate hosts or placenta of infected cows (Dijkstra et al., 2001). Bergeron et al. (2001) and Dijkstra et al. (2001) have found no oocyst shedding in a dog that have consumed naturally infected bovine foetuses and milk containing *N. caninum* tachyzoites. Little is known about sources producing infection in dogs shedding *N. caninum* oocysts in natural conditions (Basso et al., 2001a, 2001b; Slapeta et al., 2002; McGarry et al., 2003). As the oocysts of the two species *N. caninum* and *H. heydorni* whose definitive host are dogs could not be morphologically distinguished, Dubey et al. (2002) suggested their identification in isolates to be performed by a biological test and molecular methods. Through the performance of a screening study in Germany on 24 089 canine faecal samples, Scharces et al. (2005) observed oocysts similar to those of *Neospora* or *Hammondia* in 47 out of them. After performance of a biological
test and PCR, *N. caninum* oocysts were detected in only 7 isolates.

A congenital neosporosis was experimentally reproduced in bitches via subcutaneous and intramuscular inoculation of tachyzoites from tissue cultures (Dubey & Lindsay, 1989; Cole et al., 1995). The bitches remained clinically healthy, but the *N. caninum* infection could cause an early embryonic death, mummification, absorption or birth of weak puppies with neurological signs. Barber & Trees (1998) described for the first time cases of naturally acquired vertical transmission of *N. caninum* in dogs.

**Clinical signs**

Diarrhoea in dogs, accompanied by *N. caninum* oocysts shedding, was described by Blagburn et al. (1988), Basso et al. (2001) and Gondim et al. (2002).

Clinical cases of neosporosis were observed by Lindsay & Dubey (2000), Boydell & Brogan (2000) and Cantile & Arispici (2002). The heaviest form of neosporosis is encountered in young, congenitally infected dogs. Young dogs develop hindlimb paresis that advance to progressive paralysis. Other observed dysfunctions are: difficult swallowing, paralysis of jaws, muscle weakness, muscular atrophy and even cardiac troubles. The dogs with hindlimb paralysis could move and even survive for several months. The disease could be localized or generalized, with affection of all organs, including the skin. The dermatitis could be severe, caused by a huge number of *N. caninum* parasites. Four cases of skin form of neosporosis, due to mixed infection with *Leishmania* sp. (Tarantino et al., 2001; La Perle et al., 2001; Ordeix et al., 2002) are reported.

Dogs at various age could be affected. Cases of nodular dermatitis, polymyositis and multifocal encephalitis are described in dogs aged between 18 months to 6 years (Knowler & Wheeler, 1995; Pattiucci et al., 2001).

Subclinically infected bitches could transmit the parasite to their foetuses and the offspring of such bitches could be born infected. It is not known whether there is a breed or gender predisposition to neosporosis in dogs. Most reported cases are in Labrador retrievers, Boxers, Greyhounds, Golden retrievers and Bassets.

**Diagnosis**

The clinical signs shown by dogs infected with *N. caninum*, could aid to the in life diagnosis.

The dogs infected with *N. caninum* are shedding unsporulated oocysts whose size and morphology are similar to those of *Hammondia heydorni*. That is why, the use of molecular methods for differential diagnosis at this stage is mandatory (Dubey et al., 2002, Gondim et al., 2004). The blood serological analysis is also used for in life diagnosis. IFAT is the most commonly used diagnostic test. However, the detection of antibodies only by IFAT is not indicating clinical neosporosis (Lindsay et al., 1990). A small number of cross reactions between *N. caninum* and *T. gondii* are reported by Dubey et al. (1995); Trees et al. (1993) in naturally infected dogs. Significant part of dogs, shedding *N. caninum* oocysts following experimental infection, showed no seroconversion when studied for *N. caninum* by IFAT (McAllister et al., 1998; Lindsay et al., 1999a; Dijkstra et al., 2001; Schares et al., 2001a; Gondim et al., 2002). The immunoblot test against the 152 kDa antigen of *N. caninum* could however serve as an useful indicator for a preceding infection (Schares et al.,
Antibodies against *N. caninum* were also determined by iscom-ELISA from Bjorkman et al. (1994).

The post mortem diagnosis is based on the detection of parasites in lesions of affected dogs. Big lesions, characteristic for neosporosis, are CNS and liver necroses (Dubey et al., 1988a), granulomas (up to 1 cm diameter) in visceral tissues (Dubey et al., 1988a), yellowish-white strips in muscles, especially in the diaphragm (Dubey et al., 1988a; Dubey & Lindsay, 1990), cerebral atrophy (Bjerkas et al., 1984; Jackson et al., 1995) and ulcerative dermatitis (Dubey et al., 1988a; Dubey et al., 1995). For confirmation of the diagnosis, immunohistochemistry tests using specific antigens are applied.

*N. caninum* was isolated mainly from dogs showing nervous and muscle signs (Peters et al., 2000; Basso et al., 2001a).

**Prevalence**

Antibodies against *N. caninum* are reported in 121 out of 320 (37.8 %) examined dogs in Argentina (Basso et al., 2001b); in 22 % of 200 dogs in New Zealand (Reichel et al., 1998); in 10 % of 150 dogs in Turkey (Coşkun et al., 2000); in 6.7 % of 163 dogs in Brazil (Mineo et al., 2001); in 10 % of 500 domestic dogs and 25 % of 611 stray dogs in Brazil (Gennazi et al., 2002); in 6.4 % of 1058 dogs in Italy (Cringoli et al., 2002), in 12 % of 120 urban and 26 % of 81 rural dogs in Chile (Patiucci et al., 2001). Klein and Müller (2001) detected antibodies in 4 % of 50 dogs in Germany without clinical signs and in 13 % of 200 dogs with clinical manifestation. Antibodies against *N. caninum* were evidenced in 21% of 134 dogs in cattle farms in Brazil too (de Souza et al., 2002).

Comparative studies about the incidence of neosporosis among urban and rural dogs were performed. Sawada et al. (1998) detected antibodies in 31 % of 48 dogs from dairy farms and in 7 % of 198 dogs from urban areas in Japan. Wouda et al. (1998) reported a more extensive distribution in farm dogs (23.6 % of 152), than in urban dogs (5.5 % of 344) in Holland. Basso et al. (2001b) observed a higher seroprevalence in dogs from dairy farms (48 % of 125) beef-cattle farms (54.2 % of 35) than in dogs from urban regions (22.2 % of 160) in Argentina.

**NEOSPOROSIS IN COWS**

**Biological cycle**

Thilsted & Dubey (1989) were the first to discover *N. caninum*-like organism in the brain of bovine foetuses originating from a farm in New Mexico with persisting abortions. The diagnosis was definitively confirmed after obtaining a specific serum against *N. caninum* by Lindsay & Dubey (1989) that allowed to be shown that parasites in bovine foetal tissues react with antibodies against *N. caninum*.

The cows could be infected either postnatally (via horizontal transmission) or congenitally (via vertical transmission). The postnatal infection is realized via food and water, contaminated with faeces of definitive hosts that are shedding oocysts. If the cow is pregnant, the infection could be transmitted transplacentally and an abortion could follow. The vertical transmission is encountered more frequently than the horizontal one and the infection of foetus ends commonly not with abortion but with giving birth to an infected calf. The heifers originating from transplacentally infected calves could transmit the infection to the next generation when pregnant (Anderson et al.,...
The infection of cows with *N. caninum*, either natural or experimental, could result in reproductive losses. The foetuses could die intrauterinely, be absorbed, mummified, autolyzed. A preliminary delivery of living, but ill or clinically normally born but chronically infected calves could also occur (Dubey, 1999). The gestation period, during which the cow is infected, determined the outcome of pregnancy. The early infection results in absorption of the foetus, while the infection after the 6 month of gestation could end with stillbirth or congenital infection (Anderson et al., 2000).

**Clinical signs**

Clinical signs are described only in congenitally infected calves, younger than 2 months (Barr et al., 1990; Dubey & Lahunta, 1993). According to Barr et al. (1990), the clinical signs are observed most commonly 3 to 5 days after birth but could also appear later – 2 to 3 weeks after birth. The calves infected with *N. caninum* are born with a lower body weight, unviable or without clinical signs of the disease. The hindlimbs or/and the forelimbs could be bent or extremely extended. The neurological examination shows ataxia, decreased patellar reflex and lack of orientation. Sometimes, an exophthalmus, asymmetry of the eyes, hydrocephaly or narrowing of the vertebral column are observed (Dubey & Lahunta, 1993; Bryan et al., 1994).

Abortions are the principal clinical sign in cows (Dubey & Lindsay, 1996). *N. caninum* causes abortions in both dairy and meat cows, at various ages, from the 3<sup>rd</sup> month after fertilization onward. Most abortions caused by *N. caninum* occur by the 5<sup>th</sup>–6<sup>th</sup> month of gestation (Dubey & Lindsay, 1996; Anderson et al., 2000).

Although the pathophysiology of abortions caused by *N. caninum* is not well studied, the foetus is most commonly autolyzed, with placentitis and placental oedema as accompanying factors. The *N. caninum* induced abortions, rarely result in retention of the placenta or development of metritis (Dubey et al., 1996a; Dubey, 2003a).

Seropositive cows (with proved antibodies against *N. caninum*) abort more frequently than seronegative ones (Thurmond & Hietala, 1997b; Moen et al., 1998). *N. caninum* could cause repeated abortions in a small percentage (about 5%) of cows (Obendorf et al., 1995; Wouda et al., 1995; Anderson et al., 1995; Dannatt et al., 1995; Moen & Wound, 1995).

**Immunity**

Initially, the fact that the abortion could be repeated in seropositive cows, cast suspicion on the development of the protective immunity against neosporosis in natural conditions (Barr et al., 1993; Thurmond & Hietala, 1997a). Further studies showed that natural immunity against *Neospora*-induced abortion develops for a long period of time. The prevalence of abortions in acute infections (seronegative cows) is higher than those in chronic infections (seropositive dams) (McAllister et al., 2000). In the latter, the cases of abortions decrease with the number of successive pregnancies (Anderson et al., 1995; Wouda et al., 1998). These results show that some levels of immunity develop in chronically infected animals and that it is reinforced during the succeeding pregnancies.

As *Neospora* is an intracellular parasite, it could be expected that cell mediated immunity (CMI) plays an essential role. In cows, infected with *N. caninum*
(naturally or experimentally), specific antibodies and a CMI response are evidenced (Innes et al., 2002). In cows, experimentally inoculated with tachyzoites, the production of specific for the parasite CD4 + cytotoxic T-lymphocytes (CTL), able to kill cells autoinfected with *N. caninum* is shown (Staska et al., 2003). This fact also substantiates the hypothesis that the CMI response plays an important role in protective immunity. If the main protective response in naturally infected cows is cellular, the status of pregnancy could change the host-parasite balance and thus, to increase the number of parasites. In chronically infected cows, bradyzoites are activated and differentiated into infective tachyzoites thus manifesting the altered equilibrium between the host protective response and the parasite balance. Data for reactivation of *N. caninum* infection in pregnant cows are obtained by serological studies too. It is proved that in chronically infected cows, abortions are preceded by increased titres of specific anti-*N. caninum* antibodies (Dubey, 2003a). The increased antibody titres does not necessarily result in abortions. This depends on the period of gestation. The early reactivation produced an absorption, that prior to the 20th week of gestation – to abortions and after the 20th week – to premature births or birth of congenitally infected calves (Anderson et al., 1991; Barr et al., 1991). Degenerative to inflammatory lesions could be found out in foetal tissues. Most commonly they are localized in the central nervous system (CNS), the heart, skeletal muscles and liver (Barr et al., 1990; Barr et al., 1991; Wouda et al., 1998). The characteristic CNS lesions are organized as a central necrotic zone, infiltrated by inflammatory cells (glial or mononuclear). Most commonly, foetuses are autolyzed or mummified (Thornton et al., 1991; Nietfeld et al., 1992).

Epidemiology

The *N. caninum* infection in cows is most efficiently realized via vertical transmission that could be manifested in some consecutive generations (Bjorkman et al., 1996; Anderson et al., 1997; Schares et al., 1998). According to Toolan (2003) this is a very efficient means for preserving the infection within the population. The horizontal transmission is necessary for the first time during the pregnancy. Apparently, the immunization of heifers with live tachyzoites vaccine is possible, similarly to the circumstances with toxoplasmosis. Congenitally infected cows could also be preserved from abortions by challenge with live tachyzoites by the 10th week of gestation (Williams, 2001) or by vaccination with killed tachyzoites, processed with adjuvant during the first third of gestation.

Pathogenesis

Little is known about the pathogenesis of *N. caninum* lesions and the neonatal death rate in cows. Tachyzoites and tissue cysts are detected in considerable amounts in both early and later stages of gestation (Anderson et al., 1991; Barr et al., 1991). Tissue cysts prevailed in stillborn calves or calves necropsied at an age of < 7 days (Dubey & Lindsay, 1990; Barr et al., 1991). Degenerative to inflammatory lesions could be found out in foetal tissues. Most commonly they are localized in the central nervous system (CNS), the heart, skeletal muscles and liver (Barr et al., 1990; Barr et al., 1991; Wouda et al., 1998). The characteristic CNS lesions are organized as a central necrotic zone, infiltrated by inflammatory cells (glial or mononuclear). Most commonly, foetuses are autolyzed or mummified (Thornton et al., 1991; Nietfeld et al., 1992).
for introduction of a new infection in the farm (Pare et al., 1996, 1997; Thurmond & Hietala, 1997a; Wouda et al., 1998; French et al., 1998; Schares et al., 1998).

A horizontal transmission from cow to cow is not proved (Dubey, 1999). In an experiment carried out by Anderson et al. (1997), 25 seronegative heifers were reared together 25 seropositive heifers. Their progenies were examined for *N. caninum* infection. Seronegative heifers remained seronegative and gave birth to non-infected calves. Seropositive heifers remained clinically healthy but gave birth to congenitally infected calves. The necropsy of 7 congenitally infected calves revealed histological changes, characteristic for *N. caninum* infection. Guy et al. (2001) housed 3 non-infected cows together with seropositive pregnant ones, but no seroconversion was observed.

Davison et al. (2001) did not succeed to accomplish transmission of *N. caninum* in susceptible cows by feeding infected placentas.

A lactogenic transmission is experimentally evidenced in newborn calves fed with tachyzoites-infected colostrum, but there are no data for a natural infection of this kind (Davison et al., 2001).

Baillargeon et al. (2001) proved experimentally that *N. caninum* could not be transmitted venereally or via embryo transfer. The authors recommend the practical performance of embryo transfer as a means of prevention of vertical transmission.

The fact that once the cow is infected either via horizontal or vertical transmission, the protozoan persisted in its organism as a chronic infection that could be transmitted to the foetus during the gestation, is very important from epidemiological point of view (Anderson et al., 2000).

Abortions due to *N. caninum* infection are encountered all year round (Anderson et al., 1991; Thurmond et al., 1995; Moen & Wouda, 1995). In California, more cases are detected in winter than during summer or early autumn (Anderson et al., 1991). According to Moen & Wouda (1995) the abortions in Holland due to *N. caninum* infection are more common during summer than in autumn.

*N. caninum* infections in cows are evidenced in the USA (Anderson et al., 1991;1997), New Zealand (Thornton et al., 1991), Holland (Wouda, 1998), Argentina (Campero et al., 1998; Basso et al., 2001b), Belgium (de Kruif et al., 1997), Canada (Duivenvoorden & Luis, 1995), Denmark (Agerholm et al., 1997), Germany (Conraths et al., 1996), Hungary (Hornok et al., 1998), Italy (Magnino et al., 1998), Spain (Fonddevila et al., 1998), Sweden (Stenlund et al.,1997), the United Kingdom (Graham et al., 1996; Trees & Williams, 2000), Austria (Edelhofer et al., 2003), Switzerland (Gottstein et al., 1998), the Czech Republic (Vaclavek et al., 2003). The reports for the respective studies showed that 12–42 % of aborted foetuses were infected with *N. caninum* (it is known that this percentage could vary broadly – from 2.5 to 77 %). Analyzing these results, Wouda et al. (1998) stated that abortions could be either epidemic or endemic. According to Wouda et al. (1999) and Schares et al. (2002), the abortions are epidemic when more than 10 % of the risk contingent of cows abort within 6 to 8 weeks. Outbreaks of abortions with more than 30% foetal losses are communicated by McAllister et al. (1996), Neitfeld et al. (1992), Cox et al. (1998). The authors assume the possibility of a horizontal transmission of infection through concentrating of the infective agent in food and water.
Diagnosis

Several serological reactions are used for detection of antibodies against *N. caninum*: the indirect fluorescent antibody test (IFAT), the direct agglutination (DA) test and various variants of ELISA (Conraths et al., 1996; Dubey & Lindsay, 1996, Dubey et al., 1997; Jenkins et al., 1997; Wouda, 1998). The ELISA with diagnostic sensitivity of 96.4% and specificity of 96.8% is an easy and rapid method for precise detection of the *N. caninum* infection status in cows. By means of the ELISA immunoblot test, specific antibodies against *N. caninum* are successfully evidenced. Avidity ELISA, that differentiates the recent from chronic infection, could be effectively used for distinguishing endemic from epidemic abortions (Bjorkman & Uggla, 1999; Bjorkman et al., 1999). Though *N. caninum* is closely related to *T. gondii, Sarcocystis spp.* and other Apicomplexa protozoa, no cross-reactions are observed in experimental *N. caninum* infections (Dubey et al., 1996a; Wouda et al., 1998).

Some serological tests are permitted for commercial distribution: Herd Chek Idexx (Intervet); Civest Bovis Neospora Hipra (Cypress Diagnostic c.v.); Mastazyme™ (Mast Diagnostics); P38 – ELISA (Animal Welfare and Food Safety GmbH).

Von Blumroder et al. (2004) compared the serological tests in use in Europe with a standard sera pack. Most of these tests studied in various laboratories, showed a high level of compliance and very good to excellent similarity of the protocols and of data from the statistical analysis. The results show that mentioned tests could be used when performing parallel epidemiological studies. This would contribute to standardization of data interpretation.

The detection of anti-*N. caninum* antibodies in the serum of aborted cows is only indicative for contact with these protozoa and does not conform that the cause of abortion is neosporosis. Very often, sera of aborted cows show positive titres against 3 other agents: *Salmonella Dublin, Leptospira hardjo* and the bovine viral diarrhoea virus (BVDV). The negative maternal serology however is an almost certain indicator that *N. caninum* was not involved in the abortion (Wouda et al., 1998; Williams et al., 2000; Toolan, 2003).

The detection of antibodies against *N. caninum* in foetal sera and precolostrum sera from calves is indicative of infection. The negative result could not however exclude that the abortion was caused by *N. caninum* as the antibody synthesis of the foetus depends on the stage of gestation, the degree of infection and the time between the infection and the abortion (Barr et al., 1995; Wouda et al., 1997).

The ELISA is approved as a method for detection of antibodies against *N. caninum* in cow’s milk (Schares et al., 2004). It was shown that a higher number of diagnosed *N. caninum* abortions in cows is revealed by analysis of milk samples via ELISA than by the results of blood sera analysis. Andrianarivo et al. (2001) established that when infected with *N. caninum*, cows produce IgG₁ and IgG₂ antibodies in a amount, depending on the time after the infection. Soon after infection, IgG₁ antibodies are produced at higher rates than IgG₂ do. As IgG₁ is the IgG subclass present at a higher amount in cow’s milk, it could be understood why in aborted cows the ELISA, based on milk analysis was more sensitive than the tests based on blood serum analysis.

The analysis of milk samples had also better advantages than the analysis of sera,
because of the easier and cheaper collection of specimens. The milk samples are not infective. Thus, the hazard of transmission of the infection by the needle and the production losses due to stress would be eliminated.

Apart the serological analysis, the detection of the agent in tissues from aborted foetuses is needed for the definitive diagnosis of neosporosis (Dubey & Lindsay, 1996; Wouda et al., 1997). The histological investigation could be performed on brain, heart, liver, placenta or tissue fluids, but the diagnosis value is more confident when more tissues are studied. Despite that the lesions due to neosporosis are present in various organs, the foetal brain is most commonly affected. As most foetuses are autolyzed, even the semi-liquid brain tissue should be fixed in 10% buffered neutral formalin for histological examination of haematoxylin-eosin stained sections. *N. caninum* tachyzoites are immunohistochemically identified in 85% of brains, 14% of hearts and 26% of livers of 80 foetuses with confirmed neosporosis (Wouda et al., 1997). Detection of DNA of *N. caninum* via PCR could be performed in formalin-fixed, paraffin-embedded brain tissues of aborted foetuses. PCR is more sensitive for detection of *N. caninum* infection in foetuses than immunohistochemistry (Baszler et al., 1999).

**Prevention**

The prevention of horizontal transmission could be directed towards preservation of food and water for animals as well as their stores, from contamination with faeces of dogs and wild canidae. Dogs should have no access to calving premises and recently calved cows. Aborted foetuses and placentas should be collected and made harmless in a way such that the access of dogs to them is impossible (Toolan, 2003).

As in 81 to 95% of liveborn calves from seropositive dams, a vertical transmission of *N. caninum* was proved (Pare et al., 1998; Davison et al., 1999) programmes for prevention and control of this route of transmission are developed. If the prevalence in a farm is low, the culling of seropositive cows and their seropositive progeny could be feasible. In severely affected farms however, the seropositivity against *N. caninum* could be used as an additional criterion when deciding which animals should be culled and which – included in reproductive programmes. According to Wouda et al. (1998), in heavily infected farms it would be advisable to use for reproduction only seronegative heifers instead of culling numerous seropositive lactating cows. Some seropositive animals that are kept in farms could be fertilized by a beef bull and their offspring – fattened and slaughtered so that congenitally infected offspring could not be used for breeding.

For limiting the vertical transmission, Williams et al. (2000) recommended the maintenance of a closed cycle in farms. If an infected animal is introduced, the infection would not spread from it to the other animals. The purchased cow and its progeny however, would be at higher risk of abortions and congenital infections. Moreover, the infected animals in the farm are a potential source of infection for farm dogs and this could result in epidemic abortions.

That is why, the programmes for prevention during the purchase of animals include the serological test for antibodies against *N. caninum*. The use of this test would help to perform a screening of neosporosis-free animals rather than to diag-
nose abortions (Wouda et al., 1998; Williams et al., 2000).

On the basis of laboratory experiments with mice (Innes et al., 2001; Trees & Williams, 2003) the Bovilis Neogard vaccine was created (Intervet), that is licensed for production and marketing in the USA and Canada in 2001. The vaccine contains killed Neospora tachyzoites and a SPUR adjuvant. It is applied twice subcutaneously, at a 3–4 week interval, the first vaccination being done in the first third of gestation. A similar course of two injections is necessary for each following pregnancy. It is anticipated that the application of this vaccine would reduce the incidence of abortions and prevent giving birth of congenitally infected calves. The vaccine was experimentally studied in field conditions in Costa Rica (Romero et al., 2002; Frankena et al., 2004) and New Zealand (Heuer et al., 2004). A reduction of abortions with 11.2% and 24.6% respectively was reported in vaccinated cows vs non-vaccinated.

Economical losses

There are no exact data about the economical losses due to neosporosis, but they are assessed to be millions of dollars (Hoar et al., 1996). The principal economical losses in N. caninum-infected cows are related to abortions. From 20 to 43% of all abortions in cows in California (Anderson et al., 1991, 1995) and 15%–20% in Holland (Wouda et al., 1997) are due to neosporosis. For California, the annual economical losses directly associated with abortions are calculated to about $35 million.

Indirect economical losses include: expenses for professional help, diagnostics, repeated fertilization, prolonged lactation period, reduced milk and meat productivity, replacement of aborted cows if they are culled etc. (Thurmond & Hietala, 1996, 1997a, 1997b; Barling et al., 2000; Hernandez et al., 2001). In a study in California, Thurmond & Hietala (1997b) observed that seropositive cows produced daily about 1 litre milk less than seronegative. In Australia, the neosporosis impact is estimated to $ 85 millions for dairy and $ 25 millions for meat industry annually (Ellis, 1997).

These data confirm the strong need of a scientific study upon the economical importance of bovine neosporosis in other regions of the world as well.

NEOSPOROSIS IN SHEEP

Neosporosis in sheep that are intermediate hosts of N. caninum is inadequately studied. Sheep could be infected per os with N. caninum oocysts (O’Handley et al., 2002). Pregnant sheep are very susceptible to experimental infection with N. caninum tachyzoites (Dubey & Lindsay, 1990; McAllister et al., 1996; Buxton et al., 1997, 1998, 2002; Jolley et al., 1999; Innes et al., 2001). They abort dead lambs 25–30 days after the infection. Lesions in the brain, the spinal cord, the muscles and placenta are observed. An encephalitis characterized by multiple foci, hemorrhages and necroses is found out. In later stages of gestation (65, 90 and 130 days) infected sheep abort, give birth to weak or clinically normal lambs. Lesions are detected in the brain, placenta, heart, liver, lungs, even in autolyzed and mummified foetuses. Tissue cysts are observed only in the brain. The easy induction of abortions in sheep, the delivery of weak but infected lambs with or without clinical signs make sheep, on the opinion of Innes et al. (2001), an alternative model for studying bovine neosporosis. However, after postnatal infection of 3-week old lambs inocu-
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lated IV, SC or IM with tachyzoites, the animals remained clinically healthy. Therefore, that proved that such a route of infection did not result in the development of clinical neosporosis (Dubey et al., 1996a).

For the first time, Dubey & Lindsay (1990) detected N. caninum in a congenitally infected lamb in England. The lamb was born weak, with partial ataxia and died at the age of 1 week. Only tissue cysts were found out, without tachyzoites. Similar incidences of clinical neosporosis in sheep are reported in Japan. Kobayashi et al. (2001) discovered a natural neosporosis in a pregnant sheep and its twin foetuses. A focal encephalitis and thick wall tissue cysts of N. caninum were present in all three animals. N. caninum was also isolated by Koyama et al. (2001) from another lambed sheep.

Experimentally infected sheep produce antibodies against N. caninum. By the day of infection, a titre ≤ 1:50 was detected by IFAT and all sheep showed seroconversion up to ≥ 1:400 3 weeks after the infection. Little is known about seropositive infections with N. caninum in naturally infected sheep and yet, there are no data about the role of N. caninum in ovine abortions (Otter et al., 1997). The studies performed by Helmick et al. (2002) in the United Kingdom has revealed antibodies against Neospora caninum only in 3 out of 660 ewes with abortions.

NEOSPOROSIS IN GOATS

Pregnant goats are susceptible to experimental infections. N. caninum-infected pregnant goats abort infected foetuses (Barr et al., 1992; Lindsay et al., 1995).

Abortions and stillbirths are described in goats in the USA (Dubey et al., 1992), in dairy goat farms in Costa Rica (Dubey et al., 1996a) and Brazil (Corbellini et al., 2001). Antibodies against N. caninum are detected in 5 out of 77 dairy goats with abortions in Costa Rica. The percentage of seropositive goats is not known. Ooi et al. (2000) have detected no antibodies against N. caninum in 24 goats in Taiwan.

NEOSPOROSIS IN HORSES

A parasite resembling N. caninum was detected in tissues of 2 aborted equine foetuses (Dubey & Porterfield, 1990; Pronost et al., 1999), in congenitally infected foals (Lindsay et al., 1996) and 5 adult horses (Daft et al., 1997; Gray et al., 1996; Marsh et al., 1996; Cheadle et al., 1999b). Marsh et al. (1998) proposed a new name – Neospora hughesi for the parasite discovered in horses in 1996. Three N. caninum isolates from adult horses were reported by Marsh et al. (1998); Cheadle et al. (1999b); Dubey et al. (2001). Molecular and biological characterization of these 3 isolates was performed by Cheadle et al. (1999a) and Dubey et al. (2001).

The tissue cysts of N. hughesi are smaller than those of N. caninum, with a thin cyst wall (less than 1.0 µm) and with bradyzoites, smaller than N. caninum ones (Marsh et al., 1998). Thin-walled cysts characteristic for N. caninum are reported in horses from California (Daft et al., 1997) and congenitally infected foals in Wisconsin (Lindsay et al., 1996). Until now however, it is not clear whether N. hughesi is the only Neospora species that is able to infect horses. The highest prevalence of Neospora caninum in horses is observed in France 23 % (Pitel et al., 2001) and the USA: 21 %, 3 %, 17.0 % and 11.5 % in four different studies (Cheadle et al., 1999b; Dubey et al., 1999b; Vardeleon et al., 2001).
NEOSPOROSIS IN WILD ANIMALS

Wild animals could be involved in the life cycle of *N. caninum* as both intermediate and definitive hosts.

Neosporosis was diagnosed during the necropsy of 2 black tailed deers found dead in California (Woods et al., 1994), in deers from a zoo in France (Dubey et al., 1996b) and in antelopes in Germany (Peters et al., 2001). Tissue cysts of *N. caninum* are detected in the brain of a full-term stillborn deer in the Paris zoo (Dubey et al., 1996b). Peters et al. (2001) found out antibodies against *N. caninum* in amniotic fluids and DNA of *N. caninum* via PCR in brain, heart, lungs, liver and spleen or full-term antelope twins in the Hannover zoo (Germany).

The presence of antibodies in 40% of wild deers shows that the *N. caninum* cycle was probably a natural one. In a great number of these deers, the titres were high (>1:1600). The antibody titre and the prevalence percentage do not increase with age thus showing that the transmission was congenital (Dubey et al., 1999a).

Anti-*Neospora caninum* antibodies are detected in coyotes (Lindsay, et al., 1996), dingoes (Barber et al., 1997), silver and red foxes (Barber et al., 1997; Buxton et al., 1997; Lindsay et al., 2001) and wolves (Vitaliano et al., 2004). In Belgium, Shares et al. (2001b) observed a wide distribution of *N. caninum* in red foxes and proved a congenital infection. The serological results showed that wild canidae most probably play an important role in the epidemiology of neosporosis and in the life cycle of *N. caninum*.

CONCLUSION

*N. caninum* is one of the principal causes of bovine abortions in many countries all over the world. During the last decade, a great progress in the identification of the pathogen and in the knowledge about the life cycle of the parasite, the epidemiology and pathogenesis of neosporosis was achieved. So far, all possible definitive and intermediate hosts, the risk factors for the transmission, the pathogenesis of abortions caused and the measures for control and prevention of infection are not fully recognized. It is proved that the vertical transmission is the main route of infection but the elimination of vertical transmission is not sufficient for its eradication in farms as the horizontal transmission of infection is also possible. The control of *N. caninum* infections includes prevention of both vertical and horizontal transmission, including a serological study for determination of seroprevalence and excluding the seropositive animals from reproduction. The economical losses related to reduced productivity, abortions, birth of congenitally infected offspring with or without neurological signs that are a reservoir for horizontal transmission in the farm, are fairly motivating the extension of studies and control of this parasitosis.

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