Journal of Animal and Veterinary Advances 3 (12): 901-913, 2004

Bovine Neosporosis: A Review

Romero, J. J. and [1]K. Frankena
Programa de Investigación en Medicina Poblacional. Escuela de Medicina Veterinaria. Universidad Nacional, Costa Rica. P.O. Box 304-3000 Heredia, Costa Rica;[1]Quantitative Veterinary Epidemiology Group. Wageningen Institute of Animal Sciences. Wageningen University and Research Centre. The Netherlands. P.O. Box 338, 6700 AH Wageningen, The Netherlands

Introduction

Neosporosis is a parasitic disease caused by *Neospora caninum*, a protozoan that until 1988 was misdiagnosed as *Toxoplasma gondii* because of close structural similarities (Dubey, 1992; Dubey and Lindsay, 1993). This disease has been diagnosed in a wide range of hosts such as cats (Dubey *et al.*, 1990e), sheeps (Dubey and Lindsay, 1990), dogs (Dubey *et al.*, 1990b), goats (Dubey *et al.*, 1996), horses (Dubey and Portfield, 1990; Daft *et al.*, 1997) and cattle (Anderson *et al.*, 1995; Boulton *et al.*, 1995; Wouda *et al.*, 1997; Paré *et al.*, 1998; Bergeron *et al.*, 2000; Reichel, 2000; Atkinson *et al.*, 2000); causing different clinical signs. Also, it has been diagnosed, by means of serological studies, in water buffaloes (Huong *et al.*, 1998; Dubey *et al.*, 1998), camels (Hilali *et al.*, 1998), foxes and other wild canids (Barber *et al.*, 1997), and in non-human primates by means of experimental studies (Barr *et al.*, 1994; Ho *et al.*, 1997a). No antibodies to *N. caninum* have been detected in humans (Petersen *et al.*, 1999; Graham *et al.*, 1999). However, Tranas *et al.* (1999) found some evidence of seropositivity in a screening for antibodies in blood donors by indirect fluorescent antibody (IFA) tests and immunoblotting. Sixty-nine out of 1,029 (6.7%) had titers of 1:100 by IFA testing. Although the antibody titers in healthy donors were low, these data provide some evidence of human exposure to *N. caninum*.

Without doubt, most of studies has been done in cattle, and more specifically in dairy cattle, probably due to the economic impact that neosporosis has on this specie.

In some countries *N. caninum* is the main cause of abortion or foetal losses, of infectious origin, in cattle. Thilsted and Dubey described this effect for the first time at 1989. Besides, it can causes paralysis and death in dogs, and neonatal mortality and abortion in goats, sheeps and horses as well (Dubey, 1992; Lindsay *et al.*, 1996; Daft *et al.*, 1997, Dubey, 2003).

The life cycle was finally elucidated with the discovery of McAllister *et al.* in 1998. They found that the dog is the definitive host, and observed for the first time the oocysts by means of an experimental study. Further, this finding was confirmed by others (Lindsay *et al.*, 1999; Basso *et al.*, 2001). Both ways of transmission vertical (transplacental) and horizontal (postnatal), has been documented, but the vertical one is the mayor responsible for the positive animals tested (Dubey, 1999; Bjorkman *et al.*, 1996; Paré *et al.*, 1996; Thurmond *et al.*, 1997; Schares *et al.*, 1998; Davison *et al.*, 1999a,b; McAllister *et al.*, 2000; Dijkstra *et al.*, 2001).

In Costa Rica *N. caninum* was discovered in 1996 causing abortion in a goat (Dubey *et al.*, 1996a), and in 1996 it was related, for the first time, with abortion in dairy cows by means of an epidemiological study (Perez *et al.*, 1998). Other studies have proven the wide spread of the disease in the most important dairy areas of the country with within-herd seroprevalences between 10 and 88% (Romero *et al.*, 2002).

The aim of this review is to summarise, in a brief paper, the most relevant knowledge generated regarding to *Neospora caninum* and, specifically, in bovine neosporosis around the world. This review is structured as following: firstly, a general description of the agent; secondly a description of the disease with knowledge generated around the world is presented.

History: The first report of neosporosis was done under the name of Toxoplasma-like protozoan by Bjerkas *et al.* (1984). They reported encephalomyelitis and myositis in dogs between 2 and 6 months of age that showed neurological disorders; besides, found similar organisms to *Toxoplasma gondii* in lesions in the central nervous system (CNS) and muscles, but the dogs had no antibodies against *T. gondii* (Bjerkas *et al.*, 1984). In 1988, a similar parasite was found in 10 dogs in the USA, and the parasite was named *N. caninum* (Dubey *et al.*, 1988a). In retrospective studies *N. caninum* was found in dogs in the USA that died in 1957 and 1958 (Dubey *et al.*, 1988b). In 1992 Bjerkas and Dubey compared the structure and antigenicity of the parasites in fixed tissues from dogs of Norway and USA, and concluded that the parasite originally reported from Norwegian dogs, was *N. caninum* or closely related to it.

Since 1988 reports of *Neospora*-related abortions and others symptoms in cattle have been made in Europe, America, Asia, Australia and Africa (Obendorf *et al.*, 1994; Trees *et al.*, 1994; Boulton *et al.*, 1995; Jardine and Wells, 1995; Venturini *et al.*, 1995; Yamane *et al.*, 1996; Perez *et al.*, 1998).

Description of the agent

Structure: Morphological studies by electron microscopy on *N. caninum* have shown that this organism possesses a subcellular structure typical of parasites classified in the family Sarcocystidae, subclass Coccidiasina of the phylum Apicomplexa (Ellis *et al.,* 1994). This parasite is structurally very closed to *T. gondii* (Barr *et al.,* 1997) and specimens of the genus Hammondia (Hill *et al.*, 2001 and Dubey *et al.*, 2002). The known infectious stages of *N. caninum* are tachyzoites, tissue cysts and oocysts.

Tachyzoites are ovoid, lunate or round proximately 2-3 x 5-7 μm (Fig. 1). Depending on the stage of division are located in brain, myocardium, lungs and placenta of the host, but primarily in the CNS (Jardine and Wells, 1995; Dubey, 2003). In the host, tachyzoites are located within the cell cytoplasm with or without a parasitophorus vacuole, and have organelles typically found in *T. gondii* tachyzoites (Dubey, 1992). Tachyzoites have three layered plasmalema, 22 subpellicular microtubules, two apical rings, a conoid, a polar ring, one to three mitochondria, up to 150 micronemes, eight to twelve rhoptries anterior to the nucleus and four to six rhoptries posterior to the nucleus, a Golgi complex, rough and smooth endoplasmic reticulum, a nucleus and a nucleolus (Bjerkas and Prestus, 1989; Speer and Dubey, 1989; Dubey and Portfield, 1990; Barr *et al.*, 1991; Conrad *et al.*, 1993; Lindsay *et al.,* 1994; Barr *et al.,* 1997; Sonda *et al.*, 2000).

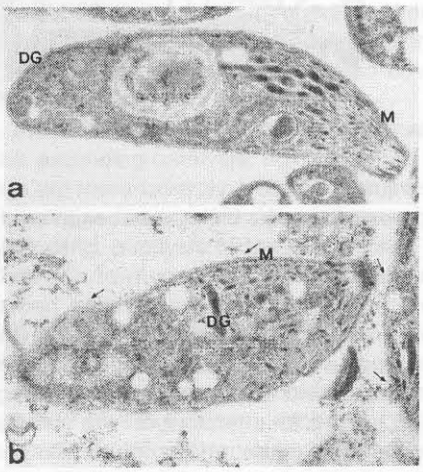

Fig. 1: Immunogold labeling EM of LR-White embedded *N. caninum* tachyzoties. Extracellular (a) and intracellular (b) tachyzoites were labeled with affinity-purified anti-recNc-p38 antibodies and secondary goat anti-mouse conjugated to 10 nm gold particles. Note the micronemes at the apical end of the cells (M) and some dense granules (DG).
Source: Sonda S. Fuchs N, Gottstein B, Hemphill A., 2000. Molecular characterization of a novel micronem antigen in Neospora caninum Mol. Biochem. Parasitol., 108: 39-51

Tissue cysts are often round to oval, up to 107 μm long and are found in several species affecting mainly the neural tissues, like brain and spinal cord (Dubey, 1992; Wouda *et al.,* 1997; Daft *et al.,* 1997). The cyst wall is smooth and up to 4 μm tick, depending upon how long infection has existed. In most tissue cysts, the cyst wall is 1-2 μm thick. Tissue cysts contain branched tubule-like structures (Bjerkas and Prestus, 1988). There is no secondary cyst wall, and septa are absent (Dubey, 1992). The cyst contains stages of the parasite called bradyzoites, that are slender structures (6-8 x 1.1.8 μm) and contains the same organelles as are found in tachyzoites except that there are fewer rhoptries and more PAS positive granules in the bradyzoites. Furthermore, tubular vesicular structures are present in between bradyzoites, which may contain micropores (Bjerkas and Prestus, 1988; Bjerkas and Dubey, 1992).

A study by Jardine (1996), with tissue cysts and bradyzoites of *N. caninum*, originated from dogs and cattle, indicated that there are not distinct ultrastructural morphological criteria differentiating *N. caninum* in dogs and cattle.

In 1998, by means of an experimental study carried out by McAllister *et al.*, it was possible to know the sexual stage of this coccidian parasite, the oocyst. Unsporulated and non-infective oocysts, of 10 to 12 μm in diameter, were observed in freshly faeces. Oocysts become sporulated and infective within 3 days outside the host. Sporulated oocysts have 2 sporocysts, each containing 4 sporozoites (Fig. 2).

There is a description of a new *Neospora* species (*N. hughesi* n. sp.), isolated from the central nervous system of an adult equine from California. The ultrastructural characteristics are very similar with *N. caninum*; however, the isolates of *N. hughesi* showed phenotypic differences in immunoreactive proteins (Marsh *et al.,* 1998).

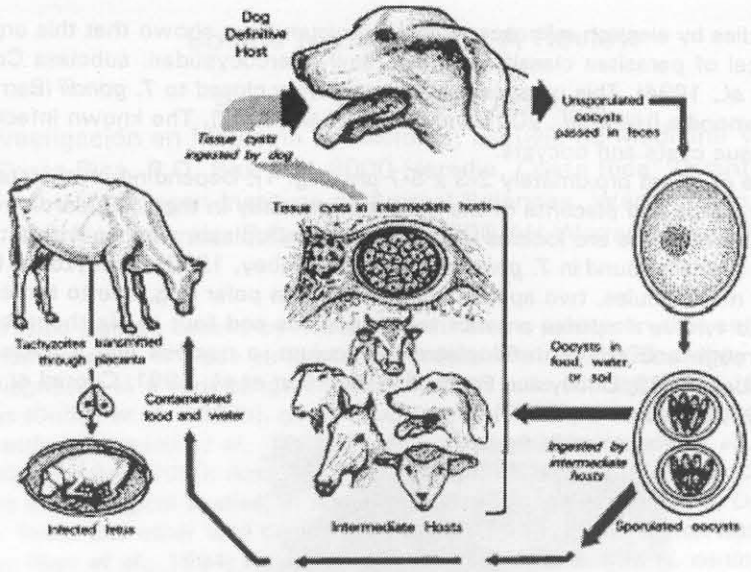

Fig. 2: Assumed life cycle of Neospora canium.
Source: Dubey, J. P., 1999. Neosporosis in cattle: biology and economic impact. J. An. Vet. Med. Assoc., 214: 1160-1163

Life cycle and transmission: Initially, it was suggested that the parasite had a life cycle like *T. gondii*, with a carnivore as definitive host (Dubey, 1992). McAllister *et al.* (1998) discovered that the dogs are the definitive host of *N. caninum.* With this finding, the life cycle was almost defined; even so, the ways of horizontal transmission remained unclear. Dubey (1999) suggest a life cycle like is shown in figure 3. Regarding to the transmission ways, the vertical transmission has been established near to the discovery of the parasite. *N. caninum* can be transmitted transplacentally in a very efficient way in dogs, cats, cattle, sheep, goats, horses and mice (Dubey *et al.*, 1990c; Barr *et al.,* 1997). Horizontal transmission has not been observed in studied species, but the some studies support this fact (Pare *et al.,* 1997; Schares *et al.,* 1998; Waldner *et al.,* 1998; Dubey, 1999; Bergeron *et al*, 2000; Romero and Frankena, 2003). The postnatal infection rates have been variable depending on the country, region of the country, the test used and the cut-off values used (Dubey, 2003). Natural infections have been diagnosed in dogs, cattle, goats, horses, sheep, deer (Barr *et al.,* 1997), buffaloes (Huong *et al.,* 1998; Dubey *et al.,* 1998) and camels (Hilali *et al.,* 1998).

In animals inoculated experimentally, *N. caninum* is infective by the subcutaneous, intraperitoneal, intramuscular and oral routes (Dubey and Lindsay, 1990; Lindsay *et al.,* 1995; Buxton *et al.,* 1997). Recent investigations conducted by Uggla *et al.* (1998) suggest that the oral infection via colostrum might be a possible route of transmission. In their experiment two neonatal calves dosed with *N. caninum* tachyzoites by feeding bottle shown DNA residues in their brain, although no pathological lesions were seen, parasites were not detected by immunohistochemistry, and was not possible re-isolate *N. caninum* of culture brain cells (Uggla *et al.,* 1998). These studies support the possibility of horizontal transmission, by means of the ingestion of oocysts in grass, water or some contaminated feed.

The possibility of venereal transmission or by embryo transfer has been tested with no clear evidence of transmission for this ways. Ortega-Mora *et al.* (2003) found DNA in fresh non-extended and frozen extended semen by real-time PCR. The parasites mean load in positive fresh semen was low (oscillating between 1 and 2.8 parasites/ml of semen). They observed *N. caninum* DNA most frequently in the cell fraction, but no specific DNA was present in the seminal fluid. These authors suggest that tachyzoites were associated to any type of cell. Based on this study, further studies are needed to determine the effect of using semen form *N. caninum*-infected bulls to inseminate heifers or cows.

On the other hand, Baillargeon *et al.* (2001) conducted a study in which seronegative cows received embryos form seropositive and seronegative donors. No any of the 70 foetuses or calves born form seronegative recipients was seropositive. Whereas, 5 out 6 seropositive recipients of embryos from seronegative donors were seropositive to *N. caninum*.

The ways through the dog acquire the parasite it is not completely clear; however, it seems to be by ingestion of contaminated material: foetuses aborted or placenta. The second one it is very likely because the parasite has been

found in naturally-infected placentas (Bergeron *et al.*, 2001) and dogs hat were fed with placentas of naturally infected cows shed *N. caninum* oocysts (Dijkstra *et al.*, 2001). Also, Basso *et al.* (2001) identified *N. caninum* oocyst by bioassay and polymerase chain reaction in faeces of naturally infected dogs.

Host-parasite Relationship

Clinical Signs: In cattle the main clinical sign is abortion between 3 and 9 months of gestational age, with a mean age of 5.6 months (Dubey and Lindsay, 1993). Also, *Neospora* may causes foetal death, retention or resorption early in gestation and, in some instances, only skeleton or mummified foetuses are aborted or retained until full gestation. Mummification appears to be an important clinical finding in outbreaks of *N. caninum* associated abortions in cattle (Nietfeld, 1992; McAllister *et al.*, 1996; Campero *et al.*, 2003). Also, it is possible the occurrence of stillborn, born alive with clinical signs, or born clinically normal but chronically infected. Seropositive cows are more likely to abort than seronegative cows (McAllister *et al.*, 1996; Perez *et al.*, 1998; Jensen *et al.*, 1999; Dubey, 2003). Abortion due to neosporosis may be endemic or epidemic (Wouda *et al.*, 1999). To these authors, abortion was considered as epidemic when more than 10% of cows at risk aborted within a period of 6-8 weeks. Repeated abortion in cows due to neosporosis is infrequent (Barr *et al.*, 1993; Anderson *et al.*, 1995). Additionally, Thurmond and Hietala (1997) reported a decreased abortion risk attributable to neosporosis in subsequent pregnancies, specially in heifers. They attributed this fact to the selective culling on cows that aborted. Subsequent abortions can be expected in those congenitally infected cows that have aborted previously, but not in high rates.

In dogs, and probably in other host, the main sign of neosporosis is severe neuromuscular disease (Dubey, 1992). Dogs infected in a natural way exhibit ascending paralysis, rigid hind limb hyperextension, which is a characteristic pattern of neosporosis. The hind limbs are more severely affected than front legs (Dubey and Lindsay, 1993; Barber and Trees, 1996). The limb cannot be flexed even with the patient under anaesthesia (Barr *et al.*, 1997). Other dysfunctions include difficulty in swallowing, paralysis of the jaw, muscle flaccidity, muscle atrophy, head tremors, forelimb ataxia, and even heart failure due to myocarditis (Dubey and Lindsay, 1992; Barber and Trees, 1996).

Lesions: *Neospora caninum* is an intracellular parasite in their forms of tachyzoites and tissue cysts, then it can causes cellular death by active multiplication of tachyzoites. Then, the lesions are caused as result of an inflammatory reaction against the parasite. *Neospora caninum* is capable of producing grossly visible lesions in a few days and destroys a variety of neural cells including those of cranial and spinal nerves (Dubey, 1992). In aborted foetuses of cattle, microscopically, multifocal non-suppurative encephalitis, myositis, myocarditis, periportal hepatitis with or without focal hepatocellular necrosis were observed repeatedly (Jardine and Wells, 1995; Danatt *et al.*, 1995; Wouda *et al.*, 1997). *N. caninum* tachyzoites have been identified immunohistochemically in brains, hearts and livers. Tissue cysts were observed in brain, spinal cord and muscles. Lesions consisting of a central focus of necrosis surrounded by inflammatory cells (glial or mononuclear) are indicative of *Neospora* infection in cattle. Only a small number of tachyzoites are present in these lesions, and they are difficult to identify without the aid of immunohistochemistry (Dubey and Lindsay, 1993).

Immune Response in the Host

Humoral Response: All studied species develops *N. caninum* IgG antibodies after 2 weeks of inoculation, being the titers higher after 3 weeks of inoculation (Dubey *et al.*, 1996b); therefore, the serologic tests are useful for detection of neosporosis infection in all species. Some different studies toward evaluating the humoral immune responses in cows, revealed a the production of both IgG1 and IgG2, in some cases with a predominance of one of them, or with a mixed response in some other cases (Andrianarivo *et al.*, 2001; De Marez *et al.*, 1999).

Specifically in cows, the immune response develops after 3 weeks with titers of 1,600 at the 21 post infection days to Immune Fluorescence Assay (IFA), and optical density ratio (OD) to ELISA greater than 0.750. At 3 months post infection, antibodies rise the highest titers. In samples of cows that aborted because of natural infection with *N. caninum*, antibody titers that ranged from 1:1,600 to 1:25,000 or more in the IFA test (Dubey *et al.*, 1996b). There was a good correlation between the antibody titer in bovine foetal fluids and the presence of lesions in the late stages of gestation (after 6 months). This correlation reflects the ability of foetuses to develop an antibody response for antigens in the later half of pregnancy (Barr *et al.*, 1997).

Reports of cows repeatedly aborting *N. caninum*-infected foetuses suggest that maternal *N. caninum* antibodies *per se* do not prevent foetal infection (Barr *et al.*, 1993; Anderson *et al.*, 1995). However, Pare *et al.* (1997) and Piergili-Fioreti *et al.* (2000) indicated that seropositive cows with high antibody levels at third trimester of gestation were less likely to abort than cows with low antibody levels at those times. Furthermore, this studies revealed that if the cow becomes infected during gestation, the foetus might not necessarily become infected. These results indicate that acquisition of infection during pregnancy is not necessary for congenital infection or abortion to occur, and suggest that maternal immune response influences congenital infection and abortion. Moreover, the foetal

immune response may be responsible for the decrease in abortion risk during the third trimester of gestation, when the foetus became immunocompetent (Osburn, 1986; Piergili-Fioreti *et al.*, 2000).

Cell-mediated Immune Response: Due to the nature of intracellular protozoan parasite of *N. caninum*, the most likely host defence against the parasite is the cell mediate immunity. Specific cell-mediated immune responses involving proliferation of cells and production of interferon (IFN) have been observed in both naturally and experimentally infected with either tachyzoites or oocysts (De Marez *et al.*, 1999; Lunden *et al.*, 1999; Andrianarivo *et al.*, 2000, Andrianarivo *et al.*, 2001).
On the other hand, some studies observed that *N. caninum* was able to produce significant amounts of IL-12 and IFN gamma, most evident shortly after infection. Also, These observations suggest that *N. caninum* induces a T-cell immune response in the infected host that is at least partially mediated by IL-12 and IFN gamma (Khan *et al.* (1997, Almeira *et al.*, 2003). Furthermore, infected dams showed a rise in lymphocyte subpopulations compared with uninfected pregnant animals (Innes *et al.*, 2001). Besides, increased levels of T lymphocytes were observed in the infected foetuses (Almeira *et al.*, 2003).
Diagnosis: In the first years form de discovery of *Neospora caninum*, the diagnosis was made by means of clinical signs and histopathology, describing lesions found in CNS, muscles and other organs of the foetuses. This kind of diagnosis had the problem that *N. caninum* was misdiagnosed like *T. gondii* due to the close structural similarities (Dubey, 1992; Dubey and Lindsay, 1993). Because this similarity, it was necessary to develop specific tests to differentiate between *N. caninum* and *T. gondii*. Thus, immunohistochemistry, serology, and molecular biology have been important in supplying specific tests to diagnose exactly, rapidly and sensitively those animals with neosporosis.

Immunohistochemistry: Bjerkas and Presthus, in the first report of Toxoplasma-like infection in dogs, used an immunohistochemical technique. However, the first immunohistochemical test specific to detect *Neospora* was an avidin-biotin-peroxidase complex immunoperoxidase staining method, developed to detect *N. caninum* in formalin-fixed paraffin-embedded tissue sections (Lindsay and Dubey, 1989). This test was able to distinguish *N. caninum* from other parasites. In the subsequent years, new immunohistochemical tests were developed to make a more specific diagnosis of *N. caninum* (Dannatt *et al.*, 1995; Barr *et al.*, 1994; Barber *et al.*, 1995; Daft *et al.*, 1997; Wouda *et al.*, 1997). This test has been used to state the presence of the parasite in tissues from foetuses suspected toward *Neospora*-induced abortion. Besides, it has been used the immunohistochemistry as gold standard test to validate serological tests (Baszler *et al.*, 2001).

Serology: As was explained above, *Neospora* induces to antibody production in the host. Then, the serological test may be useful toward detection of seropositive animals. However, a positive result to antibodies only indicates exposure to the parasite, and infection with high probability. However, histological examinations to tissues form aborted foetuses might be done to corroborate a definitive diagnosis. Even so, sometimes is not possible to identify the agent in the tissues assayed; but some lesions might be very indicative of infection to a qualified pathologist. The main serological tests used in studies for *Neospora* diagnosis have been the indirect immunofluorescent antibody test (IFAT), the enzyme-linked immunosorbent assay (ELISA) and immunoblot; however, to commercial purposes the IFAT and ELISA have been the most frequently used.
The ELISA´s have increased sensitivity and specificity for serodiagnosis of *Neospora* infection in cattle. These characteristics of the ELISA´s ranging between 88-100% and 94-96% respectively, compared with IFAT (Bjorkman *et al.*, 1994, 1997; Williams *et al.*, 1997; Romero *et al.*, 2002). In most of studies carried out during the last years, the ELISA´s have been the main test used because of facility, sensitivity and specificity in diagnosis (Pare *et al.*, 1995; Baszler *et al.*, 1996; Thurmond and Hietala, 1996; Thurmond *et al.*, 1997; Jenkins *et al.*, 1997; Osawa *et al.*, 1998). Furthermore, the ELISA has been used recently for *Neospora* diagnosis in milk, showing an agreement of 95% between serum and milk ELISA (Bjorkman *et al.*, 1997).
A very important characteristic of IFAT is its ability detecting IgM and IgG antibodies produced by the foetus in response to *Neospora* infection (Barr *et al.*, 1997). Some other tests have been validated and standardised using the IFAT as gold standard test (Paré *et al.*, 1995; Osawa *et al.*, 1998; Romero *et al.*, 2002).
Later than IFAT and ELISA, the Immunoblot tests for *Neospora* were developed, being a useful diagnostic tool to differentiate between *Neospora* and Toxoplasma species. This test, has been used to confirm the findings of ELISA and IFAT tests (Bjorkman *et al.*, 1994; Pare *et al.*, 1995; Baszler *et al.*, 1996; Hemphill and Gottsein, B., 1996 ; Beckers *et al.*, 1997; Stenlund *et al.*, 1997; Schares *et al.*, 1999).

Polymerase Chain Reaction (*PCR) Test:* Since 1993 the polimerase chain reaction (PCR) has been used to identify Neospora DNA in tissues or sera from cattle, especially to distinguish this parasite form others related with this, especially Toxoplasma (Brindley *et al.*, 1993). A study was made to identify the *N. caninum* phylogeny, which was

based on DNA sequence analysis of products derived by asymmetric PCR to determinate the nucleotide sequence. The results confirmed the placing of *N. caninum* in the family Sarcocystidae as a sister group of *T. gondii* in the phylum Apicomplexa (Ellis *et al.*, 1994). After this study, Guo and Johnson (1995) confirmed these findings, but additionally, they showed that *N. caninum* have a high level of genetic divergence with regard to *T. gondii* and other Sarcocystis species. Other studies confirm the usefulness of PCR as an important tool in specific and exact diagnosis of *N. caninum*, besides immunohistochemistry and serologic tests (Holmdahl and Mattson, 1996; Lally *et al.*, 1996; Muller *et al.*, 1996; Yamage *et al.*, 1996a,b; Ho *et al.*, 1996; Ho *et al.*, 1997a,b, Sreekumar *et al.*, 2003). Today, this technique is more and more used in epidemiological studies, even in antemortem samples (Schatzberg *et al.*, 2003). These authors developed a multiplex polymerase chain reaction (PCR) assay for the detection of Toxoplasma gondii and *Neospora caninum* DNA in canine and feline biological samples with partial results in animals with serological evidence of neosporosis or toxoplasmosis.

Treatment: Several studies have been carried out to find the best treatment for Neospora infection or its effects on the species affected, however there is currently no commercially available chemotherapy toward these goals. What is very known is the problem related to drug treatment for neosporosis in cattle is due to milk withdrawal period when is used in lactating dairy cows (Barr *et al.*, 1997). However, this problem is not very important in other species, in which the duration of chemotherapeutic treatment has lees impact. Other problem in all species is the resistance of tissue cysts and bradyzoites to killing, which does not warrantee a 100% of effectiveness in clearance of stages of the parasite in the animal (Barr *et al.*, 1997).

The sulphonamides is the main group of drugs used for *Neospora* treatment -sole or in combination with other drugs- because their historical utility in the treatment of toxoplasmosis. Then, in a study was tested the effectiveness of drugs like: Lasalocid sodium (0.05 μg/ml), monensin sodium (0.05 μg/ml), piritrexim (0.01 μg/ml), pirimethamine (0.05 μg/ml), and trimethoprim (5.0 μg/ml). These drugs were effective in preventing development of intracellular *N. caninum* tachyzoites in bovine monocyte cultured cells (Lindsay and Dubey, 1989). Authors report that treatments that combine sulphonamides with trimethoprim are able to diminish clinical signs and prevent death, but are not able to restore the health of infected animal completely (Lindsay and Dubey, 1990b; Hay *et al.*, 1990; Mathew *et al.*, 1991). In an extensive study, in which 43 chemotherapeutic agents were tested against *N. caninum* tachyzoites in cultured cells, the results indicated that some drugs have coccidiocidal activity while others coccidiostatics (Lindsay *et al.*, 1994). Besides, a study carried out by Lindsay *et al.* (1996c), demonstrated the efficacy of a treatment that combines 7 sulfonamides and 5 dihydrofolate reductase/thymidylate synthase inhibitors against tachyzoites of *Neospora caninum* in cultured cells. The better results were obtained with suboptimal concentrations of DHFR/TS inhibitors and sulfonamides.

Furher, the activity of decoquinate, an anticoccidial drug, was tested in a study, showing that acted quickly to kill intracellular stages at coccidiocidal concentrations (Lindsay *et al.*, 1997). On the other hand, using mouse models Gottstein *et al.* (2001) tested the toltrazuril and ponazuril for prevention cerebral lesion formation. Those reduced DNA detection in a PCR by >90%. The efficiency of ponazuril was also examined in infected bovines by the same author , finding that fever was reduced, humoral responses were diminished and brain pathology was also reduced (McAllister and Latham, 2001). With these findings, the researchers are now encouraged to develop efficacious chemotherapy against neosporosis in cattle.

Prevention and Control: Although now it is known the life cycle of *N. caninum*, it is difficult to make specific recommendations about preventive measures because the possibility of others canids could be definitive hosts (Barling *et al.*, 2000). Nevertheless, farmers should be encouraged to protect feed and water of faecal contamination of canine faeces (Barr *et al.*, 1997; Trees *et al.*, 1998). The weight of horizontal transmission has not been fatefully established in the total amount of new infections. However, the horizontal infection reached prevalences higher than reported initially, specially in some parts of he world (Romero and Frankena, 2003). On the other hand, the vertical transmission is the most important way of transmission (> 90%), and its control requires of other management control strategies (Trees *et al.*, 1998). As part of those strategies, culling of seropositive cows has been considered as the unique control strategy to prevent the congenital infection, but there is no evidence that this control measure has economic benefit. Therefore is necessary to assess the economic impact of neosporosis and to assess the cost of culling seropositive cows. Thurmond and Hietala (1995) recommend two general ways to prevent the *N. caninum* transmission; a) control of congenital transmission, and, b) control of postnatal transmission. This recommendations were taken into account by French *et al.* (1999) in mathematical models in which they evaluate the effectiveness of some measures of control. They assessed that selective culling (seropositives) and reduction of the possibility of vertical and horizontal transmission (control of dogs into the farm) were affective to control the infection at long term. Reduction of congenital transmission can be achieved by removing all infected cows. Furthermore, they recommend to use only seronegative heifers for replacement. The second way involves the removal of all tissue sources that are possibly infected with infective

forms of *Neospora*; e.g. placenta, foetuses, dead calves, etc., which could serve as a possible source of infection for the definitive host.

Ortega-Mora *et al.* (2003) detected *N. caninum* DNA by a real-time PCR in non-extended fresh semen samples and frozen extended semen straws of five seropositive bulls. The parasite mean load in positive fresh semen samples oscillated between 1 and 2.8 parasites/ml of semen. No *N. caninum* DNA was amplified in any of another three similar uninfected bulls (controls). On the other hand, Embryo transfer (ET) has been recommended to avoid vertical transmission of the parasite -when the embryo was received by a seronegative cow-. In this way, to evaluate efficacy of embryo transfer to prevent congenital infection some studies have been conducted, all with evidence of no congenital infection of the calves (Baillargeon *et al.*, 2001; Landmann *et al.*, 2002; Campero *et al.*, 2003). In the first one, seronegative recipients in two groups A (n = 50) and B (29) received embryos from seropositive and seronegative donors, respectively. 70 calves from groups A and B were seronegative and 9 lacked evidence of infection in tissue analysis. These results indicate that embryo transfer into seronegative recipients, could be used as an effective way to prevent vertical transmission of N caninum.

The more recent effort toward prevent Neospora-induced abortion is the vaccination. The knowledge to develop an effective vaccine and vaccination strategy against it is increasing (Hemphill *et al.*, 2000; Andrianarivo *et al.*, 1999; Innes *et al.*, 2001). Experimental studies in cattle, under laboratory and field conditions, have shown the effectiveness of several vaccinia preparations, based on killed tachyzoites with adjuvants, to elicit a response at both the cellular and humoral level (Andrianarivo *et al.*, 2000; Choromanski and Block, 2000). There is only one vaccine commercially available against the Neospora-induced abortion; but the effect of vaccination on the probability of bovine abortion and/or its effect on prevention of infection in susceptible animals have not yet been very well documented. Nevertheless, one paper reported a decreasing number of abortions after a year of vaccination using this vaccine in a Minnesota dairy herd (Choromanski *et al.*, 2001). Heuer *et al.* reported an efficacy (prevented fraction) to prevent abortion such as 0.7%, 39.0%, 54.2%, 31.4%, and -5.2%, in 5 seasonally calving, commercial dairy farms in New Zeeland. Besides, in a field trial carried out in 25 Costa Rican dairy farms by Romero *et al.* (submitted) the vaccination was associated with a 46.2% decrease of the abortion rate. However, the efficacy of vaccination to prevent *Neospora*-infection -or its consequences- in cattle is not well demonstrated until now.

Epidemiological Features: Neosporosis has been related with epizootic and sporadic abortion in dairy herds worldwide. Since the discovery of neosporosis, some studies have been conducted to assess the prevalence and to identify related factors with the disease. Prevalences have been estimated in ranges between 16.8% and70% (Pare *et al.*, 1996; Pare *et al.*, 1997; Thurmond *et al.*, 1997; Moen *et al.*, 1998; Waldner *et al.*, 1998). No specific studies have been carried out to assess incidence rates because the low odds to horizontal transmission. However, Cheryl *et al.* (1998) report apparent seroconversion rates between 6.1% and 12.6%, suggesting the odds of horizontal transmission; and in another study, Pare *et al.* (1997) report a rate of seroconversion of 8.5/100 cows/year. Romero and Frankena (2003) reported a global rate of apparent horizontal transmission such as 22%; however, they reported variations in this rate depending on the within herd seroprevalences (WHSP), increasing as the WHSP increased.

Seroepidemiological studies have been assessed the increased risk for abortion in seropositive cows (Thurmond and Hietala, 1997; Perez *et al.*, 1998; Waldner *et al.*, 1998; Wouda *et al.*, 1998a,b). Also the seropositive cows suffer higher risk of stillbirth (Waldner et la, 1998), calving a seropositive offspring (Pare *et al.*, 1997) culling for reproductive reason (Thurmond and Hietala, 1996; Waldner *et al.*, 1998) than the seronegative ones It is expected that infected cows have more odds of subsequent abortions.

There are no conclusive data that regarding economic losses due to Neospora infection anywhere in the world, but it can be estimated in millions of US dollars, due direct costs and values of the foetuses but also because of the indirect costs (Barr *et al.*, 1997; Chi *et al.*, 2002, Dubey, 2003). However, other studies have documented the effect on (re)production parameters (Barling *et al.*, 2001, Hernandez *et al.*, 2001). A reliable paper done by Trees *et al.* (1999) resumes the impact that neosporosis might produce on (re)production parameters due to several reasons.

References

Almeria, S., T. De Marez, H. Dawson, R. Araujo, J. P. Dubey, L. C. Gasbarre, 2003. Cytokine gene expression in dams and foetuses after experimental *Neospora caninum* infection of heifers at 110 days of gestation. Parasite Immunol., 25: 383-392.

Anderson, M. L., C. W. Palmer, M. C. Thurmond, J. P. Picanso, P. C. Blanchard, R. E. Breitmeyer, W. Layton, M. McAllister, B. Daft, H. Kinde, D. Read, J. P. Dubey, P. A. Conrad, B. C. Barr, 1995. Evaluation of abortions in cattle attributable to neosporosis in selected dairy herds in California. J. Am. Vet. Med. Assoc., 9: 1206-1210.

Andrianarivo, A. G., B. C. Barr, M. L. Anderson, J. D. Rowe, A. E. Packham, K. W. Sverlow, P. A. Conrad, 2001. Immune responses in pregnant cattle and bovine fetuses following experimental infection with *Neospora caninum*. Parasitol. Res., 87: 817-825.

Andrianarivo, A. G., J. D. Rowe, B. C. Barr, M. L. Anderson, A. E. Packham, K. W. Sverlow, L. Choromanski, C. Loui, A. Grace, P. A. Conrad, 2000. A POLYGEN-adjuvanted killed *Neospora caninum* tachyzoite preparation failed to prevent foetal infection in pregnant cattle following i.v./i.m. experimental tachyzoite challenge. J. Parasitol. Int. J. Parasitol., 30: 985-990.

Baillargeon, P., G. Fecteau, J. Pare, P. Lamothe, R. Sauve 2001. Evaluation of the embryo transfer procedure proposed by the International Embryo Transfer Society as a method of controlling vertical transmission of *Neospora caninum* in cattle. J. Am. Vet. Med. Assoc., 218: 1803-1806.

Barber, J. S., O. J. M. Holmdalhl, M. R. Owen, A. Uggla, A. J. Trees, 1995. Characterization of the first European isolate of *Neospora caninum* (Dubey, Carpenter, Speer, Topper and Uggla). Parasitology, 111: 563-568.

Barber, J. S. and A. J. Trees, 1996. Clinical aspects of 27 cases of neosporosis in dogs. *Vet. Rec.* 139, 439-443.

Barber, J. S., R. B. Gasser, J. Ellis, M. P. Reichel, D. McMillan, A. J. Trees, 1997. Prevalence of antibodies to *Neospora caninum* in different canid populations. J. Parasitol., 83: 1056-1058

Barling, K. S., M. Sherman, M. J. Peterson, J. A. Thompson, J. W. McNeill, T. M. Craig, L. G. Adams, 2000. Spatial associations among density of cattle, abundance of wild canids, and seroprevalence to *Neospora caninum* in a population of beef calves. J. Am. Vet. Med. Assoc., 217: 1361-1365.

Barling, K. S., J. W. McNeill, J. A. Thompson, J. C. Paschal, F. T. McCollum, T. M. Craig, T.M, Adams, L.G. 2000. Association of serologic status for *Neospora caninum* with postweaning weight gain and carcass measurements in beef calves. J. Am. Vet. Med. Assoc., 217: 1356-1360.

Barr, B. C., M. L. Anderson, J. P. Dubey, P. A. Conrad, 1991. *Neospora*-like protozoal infections associated with bovine Abortions. Vet. Pathol., 28: 110-116.

Barr, B.C., P. A. Conrad, R. Breitmeyer, K. Sverlow, M. Anderson, J. Reynolds, A. E. Chauvet, J. P. Dubey, A. A. Ardans, 1993. Congenital *Neospora* infection in calves born from cows that had previously aborted *Neospora*-infected fetuses: four cases (1990-1992). J. Am. Vet. Med. Assoc., 202:113-117.

Barr, B. C., P. A. Conrad, K. W. Sverlow, A. F. Tarantal, Hendrickx, A.G. 1994. Experimental fetal and transplacental *Neospora* infection in the nonhuman primate. Lab. Invest., 71: 236-242

Barr, B. C., J. D. Rowe, K. W. Sverlow, R. H. BonDurant, A. A. Arans, M. N. Oliver, P. A. Conrad, 1994. Experimental reproduction of bovine foetal *Neospora* infection and death with a bovine *Neospora* isolate. J. Vet. Diagn. Invest., 6: 207-215.

Barr, C., I. Bjerkas, D. Buxton, P. A. Conrad, J. P. Dubey, J. T. Ellis, M. C. Jenkins, S. A. Johnston, D. S. Lindsay, L. D. Sibley, A. J. Trees, W. Wouda, 1997. Neosporosis, Report of International *Neospora* Workshop. Comp. Con. Educ., 19: 120-126.

Bartels, C. J. M., W. Wouda, Y. H. Schukken, 1998. Risk factors for *Neospora caninum*-associated abortions storm in dairy herds in The Netherlands. Theriogenology, 52: 247-257.

Baszler, T. V., D. P. Knowles, D. P. Dubey, J. M. Gay, B. A. Mathison, T. F. McElwain, 1996. Serological diagnosis of bovine neosporosis by *Neospora caninum* monoclonal antibody-based competitive inhibition enzyme-linked immunosorbent assay. J. Clin. Microbiol., 34: 1423-1428.

Baszler, T. V., S. Adams, J. Vander-Schalie, B. A. Mathison, M. Kostovic, 2001. Validation of a commercially available monoclonal antibody-based competitive-inhibition enzyme-linked immunosorbent assay for detection of serum antibodies to *Neospora caninum* in cattle. J. Clin. Microbiol., 39: 3851-3857.

Basso, W., L. Venturini, M. C. Venturini, D. E. Hill, O. C. Kwok, S. K. Shen, J. P. Dubey, 2001. First isolation of *Neospora caninum* from the feces of a naturally infected dog. J. Parasitol., 87: 612-618.

Beckers, C. J., T. Wakefield, K. A. Joiner, 1997. The expression of Toxoplasma proteins in *Neospora caninum* and the identification of gene encoding a novel rhoptry protein. Mol. Biochem. Parasitol., 89: 209-223.

Bergeron, N., G. Fecteau, J. Pare, R. Martineau, A. Villenueve, 2000. Vertical and horizontal transmission of *Neospora caninum* in dairy herds in Quebec. Can. Vet. J., 41: 464-467.

Bjerkas, I., S. F. Mohn, J. Prestus, 1984. Unidentified cyst-forming sporozoon causing encephalomyelitis and myositis in dogs. Z. Parasitenkd, 70: 271-274.

Bjerkas, I. and J. Presthus, 1989. The neuropathology in toxoplasmosis-like infection caused by a newly recognized cyst-forming sporozoon in dogs. APMIS, 97: 459-468

Bjerkas, I. and J.P. Dubey. 1992. Evidence that *Neospora caninum* is identical to the Toxoplasma-like parasite of Norwegian dogs. *Acta Vet. Scand.* 32, 407-410.

Bjorkman, C., A. Lunden, J. Holmdahl, J. Barber, A. J. Trees, A. Uggla, 1994. *Neospora caninum* in dogs, detection of antibodies by ELISA using an iscom antigen. Parasitol. Immunology., 16: 643-648.

Bjorkman, C., O. J. M. Holmdahl, A. Uggla, 1997. An indirect enzyme-linked immunoassay (ELISA) for demonstration of antibodies to *Neospora caninum* in serum and milk of cattle. Vet. Parasitol., 68: 251-260.

Boulton, J. G., P. A. Gill, R. W. Cook, G. C. Fraser, P. A. W. Harper, J. P. Dubey, 1995. Bovine *Neospora* abortion in north-eastern New South Wales. Aust. Vet., J. 72: 119-120.

Brindley, P. J., R. T. Gazzinelli, E. Y. Denkers, S. W. Davis, J. P. Dubey, R. Jr. Belfort, M. C. Martins, C. Silveira, L. Jamra, A. P. Waters *et al.*, 1993. Differentiation of Toxoplasma gondii from closely related coccidia by riboprint analysis and a surface antigen gene polymerase chain reaction. Am. J. Trop. Med. Hyg., 48: 447-456.

Buxton, D., S. W. Maley, K. K. Thompson, A. J. Trees, E. A. Innesm, 1997. Experimental infection of non-pregnant and sheep with *Neospora caninum*. J. Comp. Pathol., 117: 1-16.

Campero, C. M., D. P. Moore, A. C. Odeon, A. L. Cipolla, E. Odriozola, 2003. Aetiology of bovine abortion in Argentina. Vet. Res. Commun., 27: 359-369.

Chi, J., J. A. VanLeeuwen, A. Weersink, G. P. Keefe, 2002. Direct production losses and treatment costs from bovine viral diarrhoea virus, bovine leukosis virus, *Mycobacterium avium* subspecies paratuberculosis, and *Neospora caninum*. Prev. Vet. Med., 55: 137-153.

Cochrane, S. M. and J. P. Dubey, 1993. Neosporosis in a Golden Retriever dog from Ontario. Can. Vet. J., 34: 232-233.

Cole, R. A., D. S. Lindsay, J. P. Dubey, B. L. Blagburn, 1993. Detection of *Neospora caninum* in tissue sections using a murine monoclonal antibody. J. Vet. Diagn. Invest., 5: 579-584.

Conrad, P. A., P. A. Barr, L. W. Sverlow, M. Anderson, B. Daft, H. Kinde, J. P. Dubey, L. Munson, A. Ardans, 993. *In vitro* isolation and characterization of a *Neospora* sp. from aborted bovine fetuses. Parasitol., 106: 239-249.

Daft, B. M., B. C. Barr, N. Collins, K. Sverlow, 1997. *Neospora* encephalomyelitis and polyradiculoneuritis in a aged mare with Cushing's disease. Eq. Vet. J., 29: 240-243.

Dannatt, L., F. Guy, A. J. Trees, 1995. Abortion due to *Neospora* species in a dairy herd. Vet. Rec., 137: 566-567.

Dijkstra, T., M. Eysker, G. Schares, F. J. Conraths, W. Wouda, H. W. Barkema, 2001. Dogs shed *Neospora caninum* oocysts after ingestion of naturally infected bovine placenta but not after ingestion of colostrum spiked with *Neospora caninum* tachyzoites. J. Parasitol. Int. J. Parasitol., 31: 747-752.

Dubey, J. P., J. L. Carpenter, C. A. Speer, M. J. Toper, A. Uggla, 1988a. Newly recognized fatal protozoan disease of dogs. J. Am. Vet. Med. Assoc., 192: 1269-1285.

Dubey, J. P, A. L. Hattel, D. S. Lindsay, M. J. Topper, 1988b. Neonatal *Neospora caninum* infection in dogs, isolation of the causative agent and experimental transmission. J. Am. Vet. Med. Assoc., 93: 1259-1263.

Dubey, J. P. and D. S. Lindsay, 1990. *Neospora caninum* induced abortion in sheep. J. Vet. Diagn. Invest., 2: 230-233.

Dubey, J. P. and M. L. Portfield, 1990. *Neospora caninum* (Apicomplexa) in and aborted equine fetus. J. Parasitol., 76: 732-734.

Dubey, J. P., W. J. Hartley, D. S. Lindsay, M. J. Topper, 1990a. Fatal congenital *Neospora caninum* infection in a lamb. Parasitol., 76: 127-30

Dubey, J. P., R. T. Higgins, J. H. Smith, T. D. O'Toole, 1990b. *Neospora caninum* encephalomyelitis in a British dog. Vet. Rec., 126: 193-194.

Dubey, J. P., A. Koestner, R. C. Piper, 1990c. Repeated transplacental transmission of *Neospora caninum* in dogs. J. Am. Vet. Med. Assoc., 197: 857-860.

Dubey, J. P, D. S. Lindsay, T. P. Limpscom, 1990e. Neosporosis in cats. Vet. Pathol., 27: 355-339.

Dubey, J. P., 1992. A review of *Neospora caninum* and *Neospora*-like infections in animals. J. Protozool. Res., 2: 40-52.

Dubey, J. P. and D. S. Lindsay, 1993. Neosporosis. Parasitol. Today, 9: 452-458.

Dubey, J. P., J. A. Morales, P. Villalobos, D. S. Lindsay, B. L. Blagburn, M. J. Topper, 1996. Neosporosis-associated abortion in dairy goat. J. Am. Vet. Med. Assoc., 15: 263.

Dubey, J. P., D. S. Lindsay, D. S. Adams, J. M. Gay, T. V. Baszler, B. L. Blagburn, P. Thulliez, 1996. Serologic response of cattle and other animals infected with *Neospora caninum*. Am. J. Vet. Res., 57: 329-336.

Dubey, J. P., S. Romand, M. Hilali, O. C. Kwok, P. Thulliez, 1998. Seroprevalence of antibodies to *Neospora caninum* and *Toxoplasma gondii* in water buffaloes (*Bubalus bubalis*) from Egypt. J. Parasitol. Int. J. Parasitol., 28: 527-9

Dubey, J. P., 1999. Neosporosis in cattle, biology and economic impact. J. Am. Vet. Med. Assoc., 214: 1160-1163.

Dubey, J. P., 2003. Review of *Neospora caninum* and neosporosis in animals. Kor. J. Parasitol., 41: 1-16.

French, N. P., D. Clancy, H. C. Davison, A. J. Trees, 1999. Mathematical models of *Neospora caninum* infection in dairy cattle, transmission and options of control. Int. J. Parasitol., 41: 464-467.

Ellis, J., K. Luton, P. R. Baverstock, P. J. Brindley, K. A. Nimmo, A. M. Johnson, 1994. The phylogeny of *Neospora caninum*. Mol. Biochem. Parasitol., 64: 303-311.

Graham, D. A., V. Calvert, M. Whyte, J. Marks, 1999. Absence of serological evidence for human *Neospora caninum* infection. Vet. Rec., 144: 672-673.

Gray, M. L., B. G. Harmon, L. Sales, J. P. Dubey, 1996. Visceral neosporosis in a 10-year-old horse. Vet. Diagn. Invest., 8: 130-133.

Gottstein, B., S. Eperon, W. Juan Dai, A. Cannas, A. Hemphill, G. Greif, 2001. Efficacy of toltrazuril and ponazuril against experimental *Neospora caninum* infection in mice. Parasitol. Res., 87: 43-48

Guo, Z. G. and A. M. Johnson, 1995. Genetic comparison of *Neospora caninum* and *Sarcocystis* by random amplified polymorphic DNA-polymerase chain reaction. Parasitol. Res., 81: 365-370.

Haile, D. G., G. A. Mount, L. M. Cooksey, 1992. Computer simulation of *Babesia bovis* (Babes) and *B. bigemina* (Smith & Kilborne) transmission by *Boophilus* cattle ticks (Acari, Ixodidae). J. Med. Entomol., 29: 246-258.

Hay, W. H., L. G. Shell, D. S. Lindsay, J. P. Dubey, 1990. Diagnosis and treatment of *Neospora caninum* infection in a dog. J. Am. Vet. Med. Assoc., 197: 87-89.

Hemphill, A. and B. Gottsein, 1996. Identification of a major surface protein on *Neospora caninum* tachyzoites. Parasitol. Res., 82: 497-504.

Hernández, J., C. Risco, A. Donovan, 2001. Association between exposure to *Neospora caninum* and milk production in dairy cows. J. Am. Vet. Med. Assoc., 219: 632-635.

Hilali, M., S. Romand, P. Thulliez, O. C. Kwok, J. P. Dubey, 1998. Prevalence of *Neospora caninum* and *Toxoplasma gondii* antibodies in sera from camels from Egypt. Vet. Parasitol., 75: 269-271

Ho, M. S., B. C. Barr, A. E. March, M. L. Anderson, J. D. Rowe, A. F. Tarantal, A. G. Hendrickx, K. Sverlow, J. P. Dubey, P. A. Conrad, 1996. Identification of bovine *Neospora* parasites by PCR amplification and specific small-subunit rRNA sequence probe hybridization. J. Clin. Microbiol., 34: 1203-1208.

Ho, M. S., B. C. Barr, A. F. Tarantal, Lai., A. G. Hendrickx, A. E. Marsh, K. W. Sverlow, A. E. Packman, P. A. Conrad, 1997a. Detection of *Neospora* from tissues of experimentally infected rhesus macaques by PCR and specific DNA probe hybridization. J. Clin. Microbiol., 35: 1740-1745.

Ho, M. S., B. C. Barr, J. D. Rowe, M. L. Anderson, K. W. Sverlow, A. E. Packman, A. E. Marsh, P. A. Conrad, 1997b. Detection of *Neospora* sp. Infected bovine tissues by PCR and probe hybridization. J. Parasitol., 83: 508-514

Holmdahl, O. J. M. and J. G. Mattson, 1996. Rapid and sensitive identification of *Neospora caninum* by in vitro amplification of the internal transcribed spacer 1. Parasitology, 112: 177-182.

Hoskins, J. D., M. M. Bunge, J. P. Dubey, D. E. Duncan, 1991. Disseminated infection with *Neospora caninum* in a ten-year-old dog. Cornell Vet., 3: 329-334.

Huong, L. T., B. L. Ljungstrom, A. Uggla, C. Bjorkman, 1998. Prevalence of antibodies to *Neospora caninum* and *Toxoplasma gondii* in cattle and water buffaloes in southern Vietnam. Vet. Parasitol., 15: 53-57

Innes, E. A., S. E. Wright, S. Maley, A. Rae, A. Schock, E. Kirvar, P. Bartley, C. Hamilton, I. M. Carey, D. Buxton, 2001. Protection against vertical transmission in bovine neosporosis. Int. J. Parasitol., 31: 1523-1534.

Jardine, J. E. and B. H. Wells, 1995. Bovine neosporosis in Zimbabwe. Vet. Rec., 137, 223.

Jardine, J. E., 1996. The ultrastructure of bradyzoites and tissue cysts of *Neospora caninum* in dogs, absence of distinguishing morphological features between parasites of canine and bovine origin. Vet. Parasitol., 62: 231-240

Jenkins, M. C., W. Wouda, J. P. Dubey, 1997. Serological response over time to recombinant *Neospora caninum* antigens in cattle after a neosporosis-induced abortion. Clin. Diagn. Lab. Immun., 4: 270-274.

Jensen, A. M., C. Bjorkman, A. M. Kjeldsen, A. Wedderkopp, C. Willadsen, A. Uggla, P. Lind, 1999. Associations of *Neospora caninum* seropositivity with gestation number and pregnancy outcome in Danish dairy herds. Prev. Vet. Med., 40: 151-163

Khan, I. A., J. D. Scwartzman, S. Fonseka, L. H. Kasper, 1997. *Neospora caninum*, role for immune cytokines in host immunity. Exp. Parasitol., 85: 24-34.

Lally, N.C., M. C. Jenkins, J. P. Dubey, 1996. Development of a polymerase chain reaction assay for the diagnosis of neosporosis using the *Neospora caninum*. Mol. Biochem. Parasitol., 75: 169-178.

Lescourret, F., J. B. Coulon, B. Faye, 1995. Predictive model of mastitis occurrence in the dairy cow. J. Dairy Sci., 78: 2167-2177.

Lindsay, D. S. and J. P. Dubey, 1989. Evaluation of anti-coccidial drugs' inhibition of *Neospora caninum* development in cell cultures. J. Parasitol., 75: 990-992.

Lindsay, D. S. and J. P. Dubey, 1990a. Infections in mice with tachyzoites and bradyzoites of *Neospora caninum* (Protozoa Apicomplexa). J. Parasitol., 76: 410-413.

Lindsay, D. S. and J. P. Dubey, 1990b. Effects of sulfadiazine and amprolium on *Neospora caninum* (Protozoa, Apicomplexa) infections in mice. J. Parasitol., 76: 177-179.

Lindsay, D. S., J. P. Dubey, S. J. Upton, R. K. Ridley, 1990. Serologic prevalence of *Neospora caninum* and *Toxoplasma gondii* in dogs from Kansas. *J. Helminthol. Soc. Washing.* 57, 86-88.

Lindsay, D. S., N. S. Rippey, R. A. Cole, L. C. Parsons, J. P. Dubey, R. R. Tidwell, 1994. Examination of the activities of 43 chemotherapeutic agents against *Neospora caninum* tachyzoites in cultured cells. Am. J. Vet. Res., 55: 976-981.

Lindsay, D. S., N. S. Rippey, T. A. Powe, E. A. Sartin, J. P. Dubey, B. L. Blagburn, 1995. Abortions, foetal death, and stillbirths in pregnant pygmy goats inoculated with tachyzoites of *Neospora caninum*. Am. J. Vet. Res., 56: 1176-1180.

Lindsay, D. S. and M. H. Birley, 1996. Climate change and malaria transmission. Ann. Tropic. Med. Parasitol., 90: 573-588.

Lindsay, D. S., H. Steinberg, R. R. Dubielzig, S. D. Semrad, D. M. Konkle, P. E. Miller, 1996b. Central nervous system neosporosis in a foal. J. Vet. Diagn. Invest., 8: 507-510.

Lindsay, D. S., J. M. Butler, N. S. Rippey, B. L. Blagburn, 1996. Demonstration of synergistic effects of sulfonamides and dihydrofolate reductase/thymidylate synthase inhibitors against *Neospora caninum* tachyzoites in cultured cells, and characterization of mutants resistant to pyrimethamine. Am. J. Vet. Res., 57: 68-72

Lindsay, D. S., J. M. Butler, B. L. Blagburn, 1997. Efficacy of decoquinate against *Neospora caninum* tachyzoites in cell cultures. Vet. Parasitol., 68: 35-40.

Lunden, A., J. Marks, S. W. Maley, E. A. Innes, 1998. Cellular immune responses in cattle experimentally infected with *Neospora caninum*. Parasite Immunol., 20: 519-526.

Marsh, A.E., Barr, B.C., Packham, A.E., Conrad, P.A. 1998. Description of a new species (Protozoa, Apicomplexa, Sarcocystidae). *J. Parasitol.* 84, 983-991.

Mathew, I. G., K. C. Smith, J. P. Dubey, L. K. Gatward, N. J. McGlennon, 1991. Treatment of encephalomyelitis due to *Neospora caninum* in a litter of puppies. J. Small Anim. Practice., 32: 609-612.

McAllister, M. M., A. M. McGuire, W. R. Jolley, D. S. Lindsay, A. J. Trees, R. H. Stobart, 1996. Experimental neosporosis in pregnant ewes and their offspring. Vet. Pathol., 33: 647-655.

McAllister, M. M., E. M. Huffman, S. K. Hietala, P. A. Conrad, M. L. Anderson, M. D. Salman, 1996. Evidence suggesting a point source exposure in an outbreak of bovine abortion due to neosporosis. J. Vet. Diagn. Invest., 8: 355-357

McAllister, M. M., J. P. Dubey, D. S. Lindsay, W. R. Jolley, R. A. Wills, A. M. McGuire, 1998. Dogs are definitive host of *Neospora caninum*. Int. J. Parasitol., 28: 1473-1478.

McAllister, D. and S. Latham, 2002. *Neospora* 2001. Trends in Parasitology . 18 (Issue 1), 4-5

Mount, G. A., D. G. Haile, R. B. Davey, L. M. Cooksey, 1991. Computer simulation of Boophilus cattle tick (Acari, Ixodidae) populations' dynamics. J. Med. Entomol., 28: 223-240.

Muller, N., V. Zimmerman, B. Hentrich, B. Gottstein, 1996. Diagnosis of *Neospora caninum* and *Toxoplasma gondii* infections by PCR and DNA hybridization immunoassay. J. Clin. Microbiol., 34: 2850-2852.

Nietfeld, J. C, J. P. Dubey, M. L. Anderson, M. C. Libal, M. J. Yaeger, R. D. Neiger, 1992. *Neospora*-like protozoan infection as a cause of abortion in dairy cattle. J. Vet. Diagn. Invest., 4: 223-226

Obendorf, D. L., N. Murray, G. Veldhuis, B. L. Munday, J. P. Dubey, 1994. Abortion caused by neosporosis in cattle. Aust. Vet. J., 72: 117-118.

Odin, M. and J. P. Dubey, 1993. Sudden death associated with *Neospora caninum* myocarditis in a dog. J. Am. Vet. Med. Assoc., 203: 831-833.

Osawa, T., J. Wastling, S. Maley, D. Buxton, E. A. Innes, 1998. A multiple antigen ELISA to detect *Neospora*-specific antibodies in bovine sera, bovine foetal fluids, ovine and caprine sera. Vet. Parasitol., 79: 19-34.

Osburn, B. I., 1986. Ontogeny of immune responses in cattle. *In* The ruminant immune system in health and disease. W.I. Morrison (ed.). Cambridge University Press, Cambridge, U.K. p: 252-260.

Pare, J., S. K. Hietala, M. C. Thurmond, 1995. An enzyme-linked immunosorbent assay (ELISA) for serological diagnosis of *Neospora* sp. Infection in cattle. J. Vet. Diagn. Invest., 7: 352-359.

Pare, J., M. C. Thurmond, S. K. Hietala, 1996. Congenital *Neospora caninum* infection in dairy cattle and associated calfhood mortality. Can. J. Vet. Res., 60: 133-139

Pare, J., M. C. Thurmond, S. K. Hietala, 1997. *Neospora caninum* antibodies in cows during pregnancy as a predictor of congenital infection and abortion. J. Parasitol., 83: 82-87.

Pare, J., G. Fecteau, M. Fortin, G. Marsolais, 1998. Seroepidemiological study of *Neospora caninum* in dairy herds. J. Am. Vet. Med. Assoc., 213: 1595-1598.

Perez, E., O. Gonzalez, G. Dolz, J. A. Morales, B. Barr, P. A. Conrad, 1998. First report of bovine neosporosis in dairy cattle in Costa Rica. Vet. Rec., 142: 520-521

Perl, S., S. Harrus, C. Satuchne, B. Yakobson, D. Haines, 1998. Cutaneous neosporosis in a dog in Israel. Vet. Parasitol., 79: 257-261

Petersen, E., M. Lebech, L. Jensen, P. Lind, M. Rask, P. Bagger, C. Bjorkman, A. Uggla, 1999. *Neospora caninum* infection and repeated abortions in humans. Emerg. Infect. Dis., 5: 278-280.

Peterson, D., K. Willard, M. Altman, L. Gatewood, G. Davidson, 1990. Monte Carlo simulation HIV infection in an intravenous drug user community. J. Acquir. Immune. Defic. Syndr., 3: 1086-1095.

Piergili-Fioretti, D., L. Rosignoli, G. Ricci, A. Moretti, P. Pasquali, G. A. Polidori, 2000. *Neospora caninum* infection in a clinically healthy calf, parasitological study and serological follow-up. J. Vet. Med. Infect. Dis. Vet. Public Health, 47: 47-53

Poli, A., F. Mancianti, M. A. Carli, M. C. Stroscio, L. Kramer, 1998. *Neospora caninum* infection in a Bernese cattle dog from Italy. Vet. Parasitol., 78: 79-85

Poncelet, L., I. Bjerkas, G. Charlier, F. Coignoul, B. Losson, Ballingand, M. 1990. Confirmation of the presence of *Neospora caninum* in Belgium. Ann. Med. Vet., 134: 501-503.

Pumarola, M., S. Anor, A. J. Ramis, D. Borras, J. Gorrais, J. P. Dubey, 1996. *Neospora caninum* in a Napolitan mastiff dog from Spain. Vet. Parasitol., 64: 315-317.

Rabinovich, J. E. and P. Himschoot, 1990. A population-dynamic simulation model of the main vectors of Chagas' disease transmission, *Rhodnius prolixus* and *Triatoma infestans*. Ecological Modelling, 52: 249-266.

Rodriguez, S. L., D. Gianola, G. E. Shook, 1997. Factors affecting susceptibility to intramammary infection and mastitis, an approximate Bayesian analysis. J. Dairy Sci., 80: 75-85.

Romero, J. J., E. Perez, G. Dolz, K. Frankena, 2002. Factors associated with *Neospora caninum* serostatus in cattle of 20 specialised Costa Rican dairy herds. Prev. Vet. Med., 53: 263-73.

Romero, J. J. and K. Frankena, 2003. The effect of the dam-calf relationship on serostatus to *Neospora caninum* on 20 Costa Rican dairy farms. Vet. Parasitol., 114: 159-71.

Sonda, S., N. Fuchs, B. Gottstein, A. Hemphill, 2000. Molecular characterization of a novel microneme antigen in *Neospora caninum*. Mol. Biochem. Parasitol., 108: 39-51.

Schares, G., M. Petersen, R. Wurm, A. Bardwald, F. J. Conraths, 1998. The efficiency of vertical transmission of *Neospora caninum* in dairy cattle analysed by serological techniques. Vet. Parasitol., 31: 87-98.

Schares, G., F. J. Conraths, M. P. Reichel, 1999. Bovine neosporosis, comparison of serological methods using outbreak sera from a dairy herd in New Zealand. Int. J. Parasitol., 29: 1659-1667.

Schatzberg, S. J., N. J. Haley, S. R. Barr, A. deLahunta, N. Olby, K. Munana, N. J. Sharp, 2003. Use of a multiplex polymerase chain reaction assay in the antemortem diagnosis of toxoplasmosis and neosporosis in the central nervous system of cats and dogs. Am. J. Vet. Res., 64: 1507-1513.

Speer, C. A. and J. P. Dubey, 1989. Ultrastructure of tachyzoites, bradyzoites and tissue cysts of *Neospora caninum*. J. Parasitol., 36: 458-463.

Sreekumar, C., D. E. Hill, V. M. Fournet, B. M. Rosenthal, D. S. Lindsay, J. P. Dubey, 2003. Detection of Hammondia heydorni-like organisms and their differentiation from *Neospora caninum* using random-amplified polymorphic DNA-polymerase chain reaction. J. Parasitol., 89: 1082-1085.

Stenlund, S., C. Bjorkman, O. J. Holmdahl, H. Kindahl, A. Uggla, 1997. Characterization of a Swedish bovine isolate of *Neospora caninum*. Parasitol. Res., 83: 214-219.

Thilsted, J. P., J. P. Dubey, 1989. Neosporosis-like abortions in a herd of dairy cattle. J. Vet. Diagn. Invest., 1: 205-209.

Thurmond, M. C. and S. K. Hietala, 1995. Strategies to Control *Neospora caninum* infection in Cattle. The Bovine Practitioner, 11: 60-63.

Thurmond, M. C. and S. K. Hietala, 1996. Culling associated with *Neospora caninum* infection in dairy cows. Am. J. Vet. Res., 57: 1559-1562.

Thurmond, M. C. and S. K. Hietala, 1997. Effect of congenitally acquired *Neospora caninum* infection on risk of abortion on subsequent abortion in dairy cattle. Am. J. Vet. Res., 58: 1381-1385.

Thurmond, M. C., S. K. Hietala, P. C. Blanchard, 1997. Herd-based diagnosis of *Neospora caninum*-induce endemic and epidemic abortion in cows and evidence for congenital and postnatal transmission. J. Vet. Diagn. Invest. 9: 44-49.

Tranas, J., R. A. Heinzen, L. M. Weiss, M. M. McAllister, 1999. Serological evidence of human infection with the protozoan *Neospora caninum*. Clin. Diagn. Lab. Immunol., 6: 765-767.

Trees, A. J., F. Guy, J. C. Low, L. Roberts, D. Buxton, J. P. Dubey, 1994. Serological evidence implicating *Neospora* species as a cause of abortion in British cattle. Vet. Rec., 134: 405-407.

Trees, A. J., H. C. Davison, A. Otter, 1998. Bovine abortion, *Neospora caninum* and dogs. Vet. Rec. 143: 343.

Uggla, A., J. P. Dubey, G. Lundmark, P. Olson, 1989. Encephalomyelitis and myositis in a boxer puppy due to *Neospora* like infection. Vet. Parasitol., 32: 255-260.

Uggla, A., S. Stenlund, O. J. Holmdahl, E. B. Jakubek, P. Thebo, H. Kindahl, C. Bjorkman, 1998. Oral *Neospora caninum* inoculation of neonatal calves. Int. J. Parasitol., 28: 1467-1472.

Veturini, L., C. di Lorenzo, C. Venturini, J. Romero, 1995. Anticuerpos anti *Neospora* sp. en vacas que abortaron. Vet. Argent, 12: 167-170.

Waldner, C. L., E. D. Janzen, C. S. Ribble, 1998. Determination of the association between *Neospora caninum* infection and reproductive performance in beef herds. J. Am. Vet. Med. Assoc., 213: 685-690.

Williams, D. J. L., J. McGarry, F. Guy, J. Barber, A. J. Trees, 1997. Novel ELISA for detection of *Neospora*-specific antibodies in cattle. Vet. Rec., 140: 328-331.

Wouda, W., J. K. Jong, Knapen, F. van., H. C. Walvoort, J. K. De Jong, 1993. *Neospora caninum* als oorzak van verlammingsverschijnselen bij jonge honden. Tijdschrift voor Diergeneeskunde. 118, 397-401.

Wouda, W., A. R. Moen, I. J. R. Visser, F. Van Knapen, 1997. Bovine foetal neosporosis, a comparison of epizootic and sporadic abortion cases in different age classes with regard to lesion severity and immunohistochemical identification of organism in brain, heart, and liver. J. Vet. Diag. Invest, 9: 180-185.

Wouda, W., A. R. Moen, Y. H. Schukken, 1998a. Abortion risk in progeny of cows after a *Neospora caninum* epidemic. Theriogenology, 49: 1311-1316

Wouda, W., C. J. M. Bartels, A. R. Moen, 1999. Characteristics of *Neospora caninum*-associated abortion storms in diary herds in The Netherlands (1995 to 1997). Theriogenology, 52: 233-245

Yamage, M., O. Flechtner, B. Gottstein, 1996a. *Neospora caninum* specific oligonucleotide primers for the detection of brain "cysts" DNA of experimentally infected nude mice by the polymerase chain reaction (PCR). J. Parasitol., 82: 272-279.

Yamane, I., T. Kokuho, K. Shimura, M. Eto, M. Haritani, Y. Ouchi, K. W. Sverlow, P. A. Conrad, 1996b. In vitro isolation of bovine *Neospora* in Japan. Vet. Rec., 138: 652.