CHAPTER II
LITERATURE REVIEW

1. Biology and life cycle of *N. caninum*

Neospora caninum is an obligate intracellular coccidian parasite belonging to the family Sarcocystidae in the Phylum Apicomplexa (Dubey et al., 1988). This parasite was first detected in the Norwegian dogs in 1984 and described in 1988 (Bjerkas et al., 1984; Dubey et al., 1988). Three stages of the life cycle of the parasite are identified as tachyzoites, tissue cyst containing bradyzoites, and oocysts (Figure 1). Tachyzoites of *N. caninum,* containing 6-16 rhoptries, are lunate, ovoid or globular and measured 3-7 x 1-5μm depending on the stage of division. Dividing tachyzoites are 4 x 3 μm (Speer and Dubey, 1989). They are located within the parasitophorous vacuole or freely in the host cell cytoplasm (Dubey et al., 2002a; Speer and Dubey, 1989).

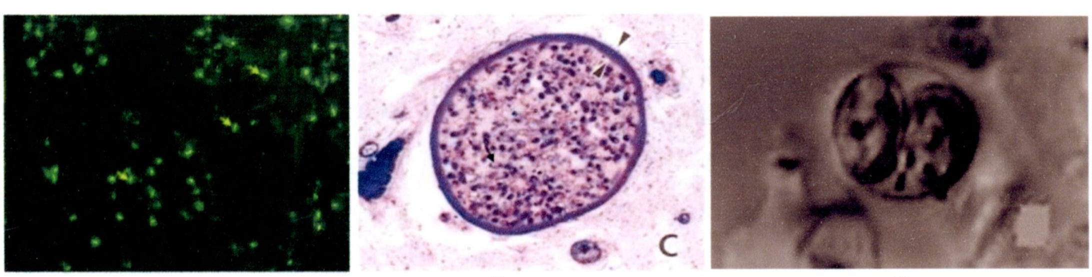

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Figure 1 Three stages of *Neospora caninum*. 1) tachyzoites (arrow heads) in an IFAT positive test (http://pcwww.liv.ac.uk/testapet/images/Scan87.jpg); 2) tissue cyst in the brain of a dog. Bradyzoites and thick wall (arrow head) (Dubey et al., 1988); 3) Oocyst (Lindsay et al., 1999b).

Round or oval tissue cysts are primarily found in the neural tissues characterized by a thick-wall up to 4μm, and the diameter up to 107 μm (Dubey et al., 2002a; Razmi et al., 2007; Zhang et al., 2007). Thin-walled (0.3-1 μm) tissue cysts were reported in the muscles of cattle and dogs naturally infected with *N. caninum*-like parasite (Peters et al., 2001a). Each neural tissue contained 50-200 bradyzoites those measured from 7.3 x 1.5 to 8 x 2 μm with 6-12 rhoptries (Dubey et al., 2002a; Speer and Dubey, 1989). Both tachyzoites and tissue cysts were found in several organs including brain, heart, kidney, liver, muscle, placenta, etc (Boger and Hattel, 2003; Dubey et al., 2006; Gibney et al., 2008; Sanchez et al., 2009; Sawada et al., 2000).

Unsporulated oocysts whose walls are colorless are reproduced from the sexual activity of the parasite and excreted with the feces by the definitive hosts. The measurements of oocyts are 10.6-12.4 x 10.6 x 12 μm with the length-width ratio of 1.04 (Lindsay et al., 1999b). One oocyst encompasses two sporocysts those are 8.4 x 6.1 μm consisting of 4 sporozoites measured about 6.5 x 2 μm in each. After being shed, oocysts sporulate within 3 days and become infective to the hosts (McAllister et al., 1998).

N. caninum has a two-host life cycle in which both sexual and un-sexual replication of the parasite takes place in the final hosts and un-sexual reproduction occurs in the intermediate hosts (Dubey et al., 2002a). As current findings, the parasite has three proven definitive hosts, i.e. dogs, coyotes and Australian dingoes. Dogs are first proven definitive host of the parasite (McAllister et al., 1998). In that study, four dogs shed *N. caninum* oocysts after being fed the infected mouse tissues. In an effort to confirm the previous finding, two mixed-breed littermate dogs were fed mouse brain containing tissue cysts of *N. caninum* isolated from beef. Both of these dogs shed oocysts on day 5 and 6 after ingesting of tissue cysts (Lindsay et al., 1999a). Since then, several researchers have reported that dogs shed *N. caninum* oocysts in their feces (Dijkstra et al., 2001; Gondim et al., 2005; Lindsay et al., 2001a; McGarry et al., 2003; Slapeta et al., 2002). The second definitive host of *N. caninum* is coyotes (Gondim et al., 2004b). The authors fed four captive-raised coyote pups with tissues from *N. caninum* infected calves, and one of the four pups excreted oocysts after feeding the tissue. Recently, experimentally infected Australian dingoes have also been reported to shed *N. caninum* oocysts (King et al., 2010).

Beside dogs, coyotes and Australian dingoes which are three known definitive host of the parasite, some other animals were trialed whether they behaved as the final hosts of *N. caninum*. Dogs and foxes were fed the tissues of a sheep and a goat experimentally infected with *N. caninum*. Both dogs and foxes excreted oocysts but the parasite found in the feces of foxes were considered not *N. caninum* (Schares et al., 2002). Similarly, studies in red-tailed hawks, turkey vultures, barn owls, and American crows failed to demonstrate their role as the definitive host of *N. caninum* (Baker et al., 1995). Interestingly, being defecated by two free ranging foxes, oocysts were morphologically and morphometrically similar to *N. caninum*, and were positive to PCR using primers targeted to Nc5 gene of *N. caninum*. In addition, DNA sequence of the isolation was 95-99% similar to the Nc5 gene (Wapenaar et al., 2006).

The most important intermediate host of *N. caninum* seems to be cattle since it causes a substantial economic loss in cattle farming. Isolations of *N. caninum* from aborted fetuses, calves and cows have been reported (Canada et al., 2004b; Fish et al., 2007; Okeoma et al., 2004b; Regidor-Cerrillo et al., 2008; Rojo-Montejo et al., 2009; Sawada et al., 2000). Buffaloes were also a vulnerable intermediate host of the parasite when *N. caninum* was demonstrated from six infected buffaloes by using bioassay and cell culture (Rodrigues et al., 2004). Furthermore, sheep, white tailed dear, red foxes, chicken and pigeons were also illustrated their role as the intermediate hosts of *N. caninum* (Almeria et al., 2002; Costa et al., 2008; Koyama et al., 2001; McGuire et al., 1999; Mineo et al., 2009; Pena et al., 2007; Vianna et al., 2005).

Rhesus monkeys have been successfully experimentally infected with *N. caninum* and induced transplacental transmission and foetal infection (Barr et al., 1994a). This discovery showed possibility of being zoonotic potential of the parasite. In addition, antibodies to *N. caninum* in human have been demonstrated (Lobato et al., 2006; Tranas et al., 1999). Fortunately, no DNA or parasite have been found in the human tissues. However, the question of the potential of whether human behaves as a host of the parasite is still unanswered. Presence of antibodies to *N. caninum* in many other species suggests that this parasite may have a wider range of intermediate hosts rather than known ones (Dubey et al., 2007a).

2. Transmission

Transmission of *N. caninum* between hosts is classified as postnatal or horizontal transmission and transplacental or vertical transmission. Horizontal transmission occurs when animals ingest tissue cysts, tachyzoites and oocysts while vertical transmission is induced when the parasites from the dams transmit to their offspring through placentas (Figure 2).

Although the parasite was first realized in Norwegian dog in 1984, according to a retrospective study, neosporosis in dogs was recorded in 1957 in four German Shepherds from Ohio (Dubey et al., 1990). In that report, there was an evidence that the congenitally infected bitches transmitted the infection to their pups. Transplacental transmission was also demonstrated in experimentally infected dogs (Cole et al., 1995; Dubey and Lindsay, 1989b). Considerably differing from cattle, vertical transmission in dog was much less effective. Only 4 out of 118 pups from 17 positive bitches were positive, and 4 out of 122 pups developed signs consistently with neosporosis (Barber and Trees, 1998). Low rate of vertical transmission suggested that there should be an effective horizontal transmission of neosporosis in dogs. However, feeding dogs with infected fetus did not always successfully induce the infection of *N. caninum*. Failure of inducing infection in the dogs fed bovine fetuses naturally infected with neosporosis was depicted (Bergeron et al., 2001; Cedillo et al., 2008). In other studies, feeding dogs with infected buffalo brains, mouse brains and calf tissues could successfully predisposed the excretion of oocysts (Gondim et al., 2002; Lindsay et al., 2001a; Rodrigues et al., 2004). Higher amount of oocysts was shed by pups and dogs fed infected calf tissues than adult dogs and dogs fed infected murine tissues, respectively. That dogs defecated oocysts is of apparentness but whether ingesting of oocysts can induce neosporosis in dog is still questionable.

Vertical transmission in cattle occurs when the parasites are transmitted from the dams to their calves through placenta, and seems to be much more effective than that reported in dogs. Studies have shown that frequency of transplacental transmission is very high in cattle from 37.1% to 95.2% (Bartels et al., 2007a; Chanlun et al., 2007; Davison et al., 1999; More et al., 2009; Pare et al., 1996; Schares et al., 1998). The efficacy of transplacental transmission in cattle depends on antibody titer and number of pregnancies. High antibody titer dams induced seropositivity in 94.8% calves while the low antibody titer dams predisposed only 14.8% seropositive offspring (More et al., 2009). The rate of endogenous transplacental infection may decrease in the subsequent pregnancies (Anderson M.L et al., 1995; Dijkstra et al., 2003; Romero and Frankena, 2003). An experiment challenging the pregnant cows with *N. caninum* oocysts successfully induced the exogenous transmission but failed to cause the endogenous transmission in the subsequent pregnancy (McCann et al., 2007).

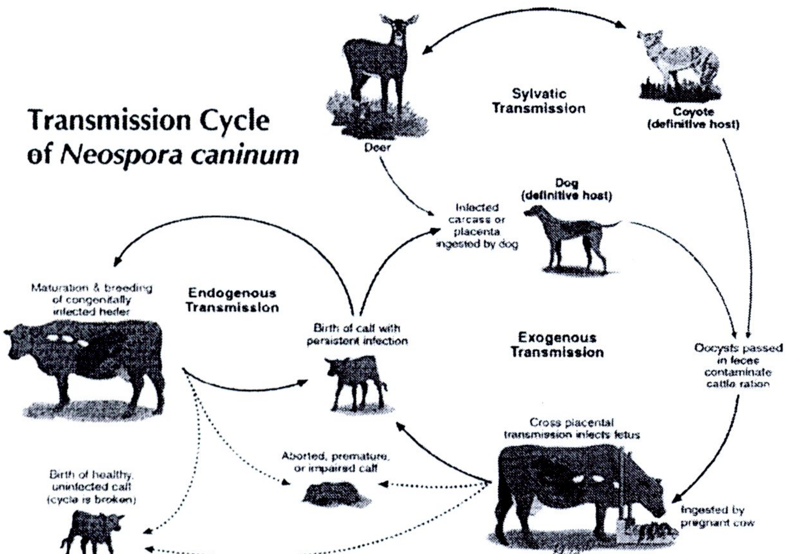

Figure 2 Life cycle of *N. caninum* (Kerry Helms, University of Illinois, College of Veterinary Medicine)

Horizontal infection of *N. caninum* occurs after birth when animals ingest oocysts excreted by the definitive hosts. The doses threshold for induction of abortion in cattle was suggested to exceed 600 oocysts (Trees et al., 2002). In Netherland, a postnatal infection rate was found very high at 47% within 6 months indicated that the herd of 95 dairy cows might have been experienced a point source exposure (Dijkstra et al., 2002). A similar result was also found in Argentina, 47% (9/19) calves showed seroconversion within 7 months (More et al., 2009). Other studies reported much lower rate of postnatal transmission from less than 1% to 5% per year (Bartels et al., 2007a; Chanlun et al., 2007; Davison et al., 1999; Hietala and Thurmond, 1999). Venereal transmission was an aroused concern since DNA of *N. caninum* was found in the semen of naturally infected bulls (Ferre et al., 2005; Ortega-Mora et al., 2003). However, the presence of parasite was low, i.e. 1-10 parasite/ml and intermittent, and the live tachyzoites in the semen have not been specified (Ferre et al., 2005). Moreover, in a study of intrauterine *N. caninum* inoculation of heifers and cows using contaminated semen, the minimum number of tachyzoites used to induce neosporosis was 50.000 (Serrano-Martinez et al., 2007b). Recently, experimentally infected bulls failed to induce seroconversion in dams through natural breeding (Osoro et al., 2009). Presence of DNA of *N. caninum* was also demonstrated in colostrum threatening the possibility of lactogenic transmission of the disease. However, live tachyzoites have not been demonstrated from milk (Moskwa et al., 2007).

Transmission of *N. caninum* has also been reported in several other animals. Vertical transmission was indicated in sheep and goat since DNA of *N. caninum* was found in the aborted fetuses (Eleni et al., 2004; Kobayashi et al., 2001; O'Handley et al., 2003). Histological finding from the aborted fetus corroborated the potential of vertical transmission in these two species (Corbellini et al., 2001; Jolley et al., 1999). Mice and cats were also suffered from neosporosis which resulted in vertical transmission (Dubey and Lindsay, 1989a; Haldorson et al., 2005; Miller et al., 2005). In a research in buffaloes in Brazil, the vertical transmission rate was demonstrated as high as 74% (Rodrigues et al., 2005). Finally, two rhesus monkeys were experimentally infected by *N. caninum* tachyzoites and predisposed transplacental transmission (Barr et al., 1994a).

3. Prevalence

N. caninum infection has been reported in several countries over the world. A considerably large number of kinds of animals from domesticated to zoo and wild, carnivorous as well as herbivorous animals have been investigated.

Dogs are an interesting subject of neosporosis studies because of its role as the definitive host of the parasite. The seroprevalence of *N. caninum* in dogs was different from continents to continents and from countries to countries. In Europe, it was reported that up to 46.4% tested dogs were seropositive to the parasite (Ferroglio et al., 2007b; Lasri et al., 2004; Wouda et al., 1999b). The proportion of positive dogs in Asia was also demonstrated from 1.2 % in Thailand to 46% in Iran (Kim et al., 2003; Kyaw et al., 2004; Malmasi et al., 2007; Ooi et al., 2000). The situation was even more serious in South America where 58.9% investigated dogs were exposed to *N. caninum* (Barber et al., 1997b; Basso et al., 2001b) which contrasted to what was explored in Oceania and Africa (Barber et al., 1997a; Wanha et al., 2005). There was a significant finding that dogs living in dairy and beef farms seemed to have higher prevalence of the disease than those did not live in the farms (Antony and Williamson, 2003; Kim et al., 2003; Sadrebazzaz et al., 2004; Sager et al., 2006; Sawada et al., 1998; Wouda et al., 1999b). This suggested that dogs play a very important role in spreading and surviving the disease in cattle farms.

Among all of animals, cattle are most studied subject in neosporosis. The individual prevalence of *N. caninum* in cattle varied widely ranging from as low as 0% to as high as 87 % (Akca et al., 2005; Stenlund et al., 2003). Reports frequently showed a prevalence of less than 30% (Dubey et al., 2007a). Dairy cattle seemed to have higher prevalence of *N. caninum* compared with beef cattle (Bartels et al., 2006a; Dubey et al., 2007a; Koiwai et al., 2005b). Almost all studies stated an individual seropositive status of less than 30% in beef cattle except one which found 79% aborted beef positive in America (Dubey et al., 2007a; McAllister et al., 2000). Herd level prevalence was also reported variously between 16% and 94% (Bartels et al., 2006a; Woodbine et al., 2008).

Buffaloes have also been reported vulnerable to neosporosis. Similar to what found in dogs and cattle, prevalence of *N. caninum* in buffaloes also varied between countries and countries, from as low as 0% up to 70.9% (Dubey et al., 1998; Fujii et al., 2001; Gennari et al., 2005; Guarino et al., 2000; Huong et al., 1998; Mohamad et al., 2007; Rodrigues et al., 2005; Yu et al., 2007) (Figure 3). When attention to subtypes of water buffaloes is paid, there may be an interesting difference between them in the aspects of the tolerance to *N. caninum*. River buffaloes seemed to have a higher seropositivity than swamp buffaloes. Reports conducted in river buffaloes from Italy, Egypt, Argentina, Brazil and India depicted a very high prevalence between 34.6% and

70.9% (Campero et al., 2007; Dubey et al., 1998; Gennari et al., 2005; Guarino et al., 2000; Meenakshi et al., 2007; Mohamad et al., 2007). By contrast, seroprevalence in swamp buffaloes In Vietnam, China and the Philippines was very low, from 0% to 2.0% (Huong et al., 1998; Konnai et al., 2008; Yu et al., 2007).

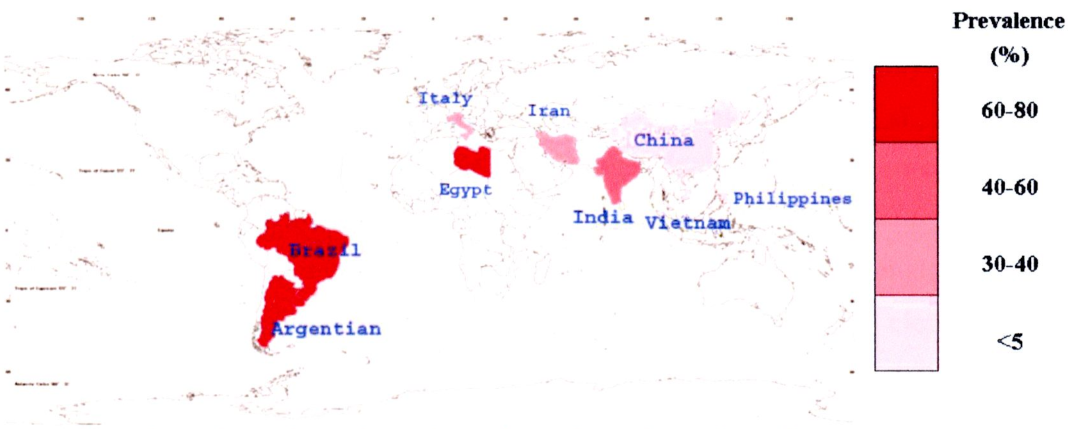

Figure 3 Neosporosis map in water buffaloes

Prevalence of neosporosis in several other animals has also been published. In sheep and goat the proportions of seropositive experimented subjects have been found up to 26.3% (Konnai et al., 2008). A wide range of wild carnivorous, herbivorous, zoo and marine animals have been studied the prevalence of *N. caninum* (Almeria et al., 2007; Dubey, 2003; Panadero et al., 2010; Sedlak and Bartova, 2006; Sobrino et al, 2008; Steiman et al., 2006).

The possible zoonotic aspect of *N. caninum* has been a concern due to the demonstration of antibodies against this parasite in human. *N. caninum* seropositive people were reported in Brazil (Lobato et al., 2006). In that study, 38% and 18% people who had acquired immuno-deficiency syndrome and neurological disorders were respectively positive to *N. caninum*. Testing 1,029, 247 and 172 blood donors in the United States, Northern Ireland and Korea, the results showed that 6.7%, 5.3% and 6.7% samples were positive, respectively (Graham et al., 1999; Nam et al., 1998; Tranas et al., 1999). For review of prevalence of *N. caninum* infection: see Dubey et al. (2007a).

4. Clinical signs

Neosporosis have been reported in dog at different ages with the most found clinical signs of locomotor ataxia, paresis or paralysis of either hindlimbs or forelimbs, or both (Barber and Trees, 1996; Basso et al., 2005; Crookshanks et al., 2007; Dubey, 2005, 2007a; Peters et al., 2000). The hindlimbs were usually more severely affected than the forelimbs (Dubey et al., 2007a). Other dysfunctions may include rigidity of the legs (Reichel et al., 1998), muscle atrophy, stiff jaws and dysphagia (Basso et al., 2005), rigid hyperextension (Barber and Trees, 1996) and Horner's syndrome (Mayhew et al., 2002). Multifocal nodular dermatitis, ulcerative and pyogranulomatous dermatitis were also found in the dogs infected with *N. caninum* (Boyd et al., 2005; La Perle et al., 2001; Perl et al., 1998). How the dogs developed neuromuscular symptoms was not clear but it was most likely that they were affected by the damage in the central nerve system such as cerebella atrophy, multifocal non-suppurative encephalitis (Dubey, 2005; Lorenzo et al., 2002), multifocal non-suppurative meningoencephalomyelitis (Patitucci et al., 1997), myeloencephalitis (Pumarola et al., 1996) and myositis (Crookshanks et al., 2007; Paciello et al., 2004; Pasquali et al., 1998).

In the adult cattle infected with *N. caninum*, abortion was the only demonstrated clinical sign (Armengol et al., 2006; Kul et al., 2009; Razmi et al., 2007; Reiterova et al., 2009). Infection of *N. caninum* at the early stage of gestation may result in fetal death, resorption or mummification (Ghanem et al., 2009; Williams D.J et al., 2000) while exposure to the parasite at later stage may result in either calving with congenital infected calves or abortion (Innes et al., 2001; McCann et al., 2007; Rosbottom et al., 2008; Williams D.J et al., 2000). Abortion due to neosporosis was documented to occur through the period of gestation but the majority was between 5th and 7th month of pregnancy (Huang et al., 2004; Wouda et al., 1997). *N. caninum*-positive cattle had higher risks up to 22.3 times of being aborted than their neosporosis negative counterparts (Lista-Alves et al., 2006; Lopez-Gatius et al., 2004a; Moore et al., 2009; Oshiro et al., 2007; Weston et al., 2005). Within herds, abortion can be considered as epidemic or endemic abortion. In the case of epidemic abortion the rate of fetus loss can be up to 10% pregnancies or more in a short time while endemic abortion scatters throughout the year with lower rate (Collantes-Fernandez et al., 2006a; Crawshaw and Brocklehurst, 2003). In several herds, the positive animals could be aborted from 13% to 44% of the pregnancies (Dijkstra et al., 2001a, 2002; Lopez-Gatiuz et al., 2004a; McAllister et al., 2000; Schares et al., 2002a). Abortion occurred not only in one pregnancy but also in subsequent gestations with lower rate (Pabon et al., 2007; Stahl et al., 2006; Thurmond and Hietala, 1997a).

Although most of the calves born from the positive dams were congenitally infected, majority of them were clinically healthy and some expressed the abnormal clinical signs (Pare et al., 1996). Infected calves may be born underweight, unable to rise and with the neurological signs. Either hindlimbs and/or forelimbs could be flexed or hyperextended. The neurological examination revealed ataxia, decreased patellar reflexes and loss of conscious proprioception (Barr et al., 1993; Parish et al., 1987). The clinical signs in the infected calves may be due to the pathological findings including lesions in brain characterized with non-supportive necrosis foci, focal necrotizing encephalitis, non suppurative encephalomyelitis, non suppurative myositis, myocarditis (De Meerschman et al., 2002; Pescador et al., 2007; Razmi et al., 2007; Zhang et al., 2007).

Clinical signs of neosporosis have also been reported in several other animals. Abortion caused by *N. caninum* was found in buffaloes, sheep, goats and pigs with variety of systematic disorders of the fetus including myocarditis, myositis, pneumonitis, nephritis, hepatitis and encephalitis (Buxton et al., 1998, 2001; Dubey et al., 1996b; Guarino et al., 2000; Jensen L et al., 1998; McAllister et al., 1996b). In addition, meningoencephalomyelitis and myeloencephalitis were found in deer and horses, respectively (Marsh et al., 1996; Soldati et al., 2004). Moreover, rhinoceros and antelopes were infected with *N. caninum* with symptoms such as myocarditis and stillbirth, respectively (Peters et al., 2001b; Williams J.H et al., 2002). Recently, experiments to infect chicken and embryonated eggs with *N. caninum* have induced arthritis in the feet joint of chicken and death of embryonated eggs (Furuta et al., 2007; Mansourian et al., 2009).

5. Pathogenesis of abortion in animals

In cattle, abortion was defined as the termination of pregnancy between day 42th and 260th of gestation (Lopez-Gatius et al., 2004b). For abortion to occur, the fetus and/or placenta must be damaged so that they are no longer viable. It is still unclear how the parasite causes the abortion but there are several possible explanations for this phenomenon including the direct effect of fetal tissue damage affected by the multiplication of the parasite, and the response of the maternal and foetal immunities to the parasite which resulted in death of placental tissue and subsequent insufficiency of oxygen and/or nutrition (Dubey et al., 2006).

Cattle embryos within 7 days of gestation did not expose to the parasite in the positive dams (Landmann et al., 2002; Moskwa et al., 2008). From day 34th to 90th of pregnancy, there was not any association between abortion and seropositivity to *N. caninum* (Lopez-Gatius et al., 2004b). However, it did not mean that in this stage the parasite did not infect the fetuses since there was evidence that neosporosis increased the number of services per conception in cattle (Hall et al., 2005). In later period of gestation, infection of *N. caninum* might result in fetal death or congenitally infected progenies. The time of infection seemed crucial to the outcome of the disease when challenging the pregnant cows with *N. caninum* tachyzoites at day 70th of gestation resulted in fetal death while infection at day 210th conferred the transplacentally infected calves (Rosbottom et al., 2008; Williams D.J et al., 2000). In the cows infected at day 70th, widespread necrosis and inflammation in the placentas were found while those pathological symptoms were absent in the group of cows infected at day 210th (Rosbottom et al., 2008). Before about day 100th of gestation the fetus could not recognize and respond to the pathogens (Osburn et al., 1982) then the parasite could easily invade and multiplicate. The parasite could reinvade the placentas from the fetus and caused more severe necrosis in the placentas (Gibney et al., 2008). As the result, the fetus might be dead due directly to the destruction of the parasite or the cytotoxic effects of the necrosis process that damaged the trophoblast cells. Furthermore, there was a speculation that the infection of bovine neosporosis in the first trimester may induce the T helper cell-1 cytokines response and lead to the generation of IL-12, IFN-γ and TNF-α and subsequent production of free oxygen radicals such as nitric oxide, all of which may be lethal to parasite but may also kill the fetus (Quinn et al., 2002). That is why the infection of *N. caninum* in the first trimester usually resulted in severe pathogenesis in the placenta and death of fetus (Barr et al., 1994b; Buxton et al., 1998; Dubey et al., 1992; Rosbottom et al., 2008).

After day 100^{th} of gestation the immune system of the fetus is competent to recognize and respond to the antigens (Osburn et al., 1982). However, the abortion due to neosporosis peaked during month 5^{th} -7^{th} of gestation (Gonzalez et al., 1999; Moen et al., 1998; Thurmond and Hietala, 1997a; Thurmond et al., 1997). Challenging of pregnant cows with *N. caninum* oocyst at different stages of gestation resulted in abortions at group infected at day 120^{th} of pregnancy while there were no abortions in groups inected at day 70^{th} and 210^{th} (Gondim et al., 2004a; McCann et al., 2007). This may be explained by the pattern of progesterone in the gestation of the cattle which increased steadily from early to mid-gestation then significantly declined few weeks before parturition (Pope et al., 1969). Supplementation of progesterone at mid-gestation increased the risk of abortion in *Neospora*-infected dairy cows with high antibody titer was reported (Bech-Sabat et al., 2007). In addition, the peak response of the cell mediated immunity (CMI) to the parasite was at the early and late gestation when the level of progesterone was low (Innes et al., 2001). In other words, CMI responded to parasite less effectively at the mid-gestation than at first and third trimesters. When the immune response of mother changed to facilitate the pregnancy, it might also favour the multiplication of the parasite. As the result, the modulation of the CMI might influence the recrudescence of a previous persistent infection causing bradyzoites to excyst resulting in parasitaemia (Innes et al., 2001). Another suggestion was that as the pregnancy progressed to mid-gestation, the parasite would have sufficient time for further implication (Lopez-Gatius et al., 2004b). Those hypotheses may explain why abortion peaked between month 5^{th} and 7^{th} of gestation.

In the third trimester, infection of *N. caninum* usually predisposed persistently infected progeny, otherwise healthy calves (McCann et al., 2007; Rosbottom et al., 2008).

Immuno-competence was very important to the survival of the fetus (Williams D.J et al., 2000). In an experiment, inoculating tachyzoites to 2 groups of pregnant cows at 10^{th} and 30^{th} weeks of pregnancy, an increase in response of Th1 to the presence of parasites was observed in both groups (Williams D.J et al., 2000). Despite this fact, fetal death occurred only in the former group, in the latter group calves were born congenitally infected. There was a suggestion that the response of Th1 might be too late to affect an existing, well-established Th2 response at maternal-fetal interface. T helper 1 cytokines facilitated the pro-inflammatory cytokines which effectively killed the infected cells and parasites while T helper 2 cytokines worked less effectively than the former (Quinn et al., 2002). Throughout the gestation, the ratio of Th2:Th1 increased because of the production of Th2 from the fetal tissue (Wegmann et al., 1993). The modulation of response of Th2 may result in less necrosis and inflammation in the placenta and fetus but favour the survival of parasite and its invasion to the fetus and subsequent congenitally infected offspring (Williams D.J et al., 2000).

6. Risk factors of neosporosis

6.1 Risks of infection

There were certain factors that contributed as risks of infection in neosporosis. Studies of canine neosporosis showed that seroprevalence of *N. caninum* was higher in dogs in rural areas than that in dogs living in urban areas (Ferroglio et al., 2007b; Hornok et al., 2006a; Sharma et al., 2008). Farm dogs were more likely to be positive than urban dogs, house dogs and rescue dogs (Cruz-Vazquez et al., 2008; Hornok et al., 2006a; Paradies et al., 2007). This may be explained that dogs living in rural areas and the farms more easily accessed to the source of *N. caninum* such as aborted fetus or infected placentas. Due to postnatal transmission, age had a positive correlation with the *N. caninum* sero-status in dogs (Malmasi et al., 2007). Climate condition might influence the development of *N. caninum* oocysts then affect the seroprevalence of animals living in that region. In Spain, carnivores living in high humid areas had higher prevalence of antibodies to the parasite (Sobrino et al., 2008).

The presence of dogs in the farm increased the risk of being positive to *N.*

caninum of cattle (Bartels et al., 2007a; Gonzalez-Warleta et al., 2008). Rabbits and ducks were also a putative risk factor for the seropositivity of cattle (Ould-Amrouche et al., 1999). The risk of being positive increased with the age and parity of the cattle for the existence of horizontal transmission (Dyer et al., 2000; Jensen A.M et al., 1999; Rinaldi et al., 2005; Sanderson et al., 2000; Taubert et al., 2006). Seroprevalence to *N. caninum* was also different from breeds to breeds when Limousin was reported to have lower sero-status compared with other breeds (Armengol et al., 2007). In the same raising condition, dairy cattle seemed to be more vulnerable to *N. caninum* than beef cattle (Bartels et al., 2006a; Jensen A.M et al., 1999; Moore et al., 2009). Infection of Infectious Bovine Rhinotracheitis was reported to predispose seropositivity of *N. caninum* in cattle since the former disease harmfully affected the immune system of the cattle and created opportunity for the latter pathogen to infect animals (Rinaldi et al., 2007). It was likely to be true that the higher antibody titer cattle would have more chances to transmit the infection to their calves than the cattle had lower antibodies titer (More et al., 2009). In the water buffalo, age and sex were reported to have influence on the seroprevalence of the animals when older and female buffaloes had higher risk of being positive than the younger and male buffaloes, respectively (Campero et al., 2007; Guarino et al., 2000; Mohamad et al., 2007).

6.2 Risks of abortion

Seropositive cows were more likely to abort than their negative counterparts (Gonzalez-Warleta et al., 2008; Ica et al., 2006; Lista-Alves et al., 2006; Lopez-Gatius et al., 2007a, 2007b; Moore et al., 2009; Weston et al., 2005). The abortion risk increased with the increasing levels of *N. caninum* antibodies in individual animals (Kashiwazaki et al., 2004; Stenlund et al., 2003; Waldner, 2005). Herds with high prevalence *N. caninum* antibodies were associated with increased risk of abortion (Bartels et al., 2006b; Fischer et al., 2003; Hobson et al., 2005; Schares et al., 2004). Age and parity of cattle were found to be protective factors of abortion (Hernandez et al., 2002; Stahl et al., 2006). The presence of other animals in the farm contributed as either risk or protective factors to the abortion of cattle. Farms with the presence of dogs and horses were reported to have higher rate of pregnancy termination whereas the presence of cats in farms could decrease the abortion rate of dairy cattle (Hobson et al., 2005). Perhaps, cats had interrupted the transmission of parasites from intermediate hosts like rats to dogs by eating them or replaced the presence of the dogs in the farms then decreased the dissemination of the parasites and abortion of cattle. The risk of abortion was 15.6 times higher in the cows did not produce IFN-γ than sero-negative cows why neosporosis had no effects on the seropositive cows that produced IFN-γ (Lopez-Gatius et al., 2007a). High levels of prolactin had protective effect on the abortion rate caused by neosporosis while supplementation of progesterone in the mid-gestation of high antibody titer cattle increased the abortion rate (Bech-Sabat et al., 2007; Garcia-Ispierto et al., 2009). High humidity climate was also a risk of abortion. The suggestion was that the humid environment favored oocysts to sporulate and infect and cause abortion in cattle (Wouda et al., 1999a).

7. Diagnosis of neosporosis

A certain many techniques have been used to diagnose *N. caninum* in animals. Researchers have isolated and demonstrated viable parasites from infected animals, studied the lesions caused by the parasites in the infected tissues, found the DNA and tested the seroprevalence of *N. caninum* in a very wide range of animals and samples.

7.1 Cell culture and bioassay

Isolation of viable tachyzoites by cell culture and bioassay are definitive methods to demonstrate the infection caused by *N. caninum* (Figure 4). However, these two methods were not always successful (Dubey and Schares, 2006). Despite of low achievements of the approaches because the autolysis of tissue may kill the parasites in the samples, several isolations in a number of animals have been demonstrated.

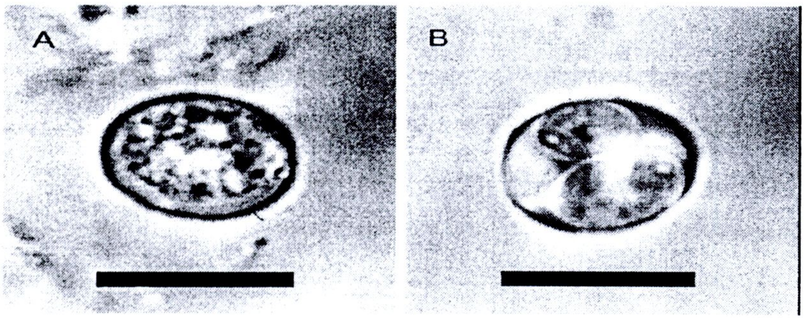

Figure 4 *Neospor*ulated ooc*a caninum* oocysts shed by a coyote (*Canis latrans*). Bars = 10 mm. (A) Unsporulated oocyst. (B) Sporyst containing two sporocysts (Gondim et al., 2004)

Knock out mice, nude mice, cortisonized mice, gerbils, dogs and Vero cell monolayer have been used to successfully isolate viable *N. caninum* from cattle, buffaloes, white tailed-deer, dogs and sheep (Basso et al., 2001a; Dubey et al., 2007b; Gondim et al., 2001; Pena et al., 2007; Regidor-Cerrillo et al., 2008; Rodrigues et al., 2004; Rojo-Montejo et al., 2009; Vianna et al., 2005). Brains of the infected animals seemed to be the most suitable samples (Canada et al., 2004b; Cheah et al., 2004; Fish et al., 2007; Okeoma et al., 2004b; Sawada et al., 2000). In addition, feces and lesions of infected dogs were also used as candidate specimens (Basso et al., 2009; McInnes et al., 2006a).

7.2 Histopathology and immunohistochemistry

Histopathology and immunohistochemistry are two diagnostic tests usually used to examine the pathomorphological changes caused by infection (Jenkins et al., 2002; Thurmond et al., 1999). Lesions characterized by multiple foci of non-suppurative infiltrates and necrotic areas in the tissues and focal necrosis without a cellular reaction and focal gliosis without necrosis in brain (Collantes-Fernandez et al., 2006a) were the targets in the specimens of which brains and spinal cords were the most common tissues followed by hearts, lungs, kidneys, placenta, skeleton and livers of the aborted fetuses and infected dams (Dubey and Schares, 2006; Kul et al., 2009; Pabon et al., 2007; Sanchez et al., 2009) (Figure 5). Higher parasites burdens and lesion rates were observed in epidemic abortion than endemic abortion, especially when comparing those in the hearts and livers of the aborted fetuses (Collantes-Fernandez et al., 2006a). Similarly, the severity of the lesions was affected by the stage of pregnancy when they were more severe in the first and second trimester than those in the trimester (Collantes-Fernandez et al., 2006b) The lesions might result in encephalomyelitis, myocarditis, hepatitis, encephalitis, meningoencephalitis, epicarditis, placentitis and myositis found in the histopathologically analyzed samples (De Meerschman et al., 2002; Dubey and Schares, 2006; Moore et al., 2002; Pescador et al., 2007; Razmi et al., 2007; Zhang et al., 2007). Beside examination of the lesions, the method looked for tachyzoites and tissue cysts containing bradyzoites as well (Barr et al., 1991; Guarino et al., 2000). To confirm to results of histological method, immunohistochemistry using peroxidase-labeled *N. caninum* antiserum was applied (Kyaw et al., 2005; Moore et al., 2002). Furthermore, the latter approach could be used to distinguish not only tachyzoites from bradyzoites but also *N. caninum* from other related parasite such as *T. gondii* (Barr et al., 1991; Lindsay and Dubey, 1989; McAllister et al., 1996b; Otter et al., 1995). This method is highly specific but not very sensitive due to the autolysis of the samples and the unequal distribution of the parasites in the specimens.

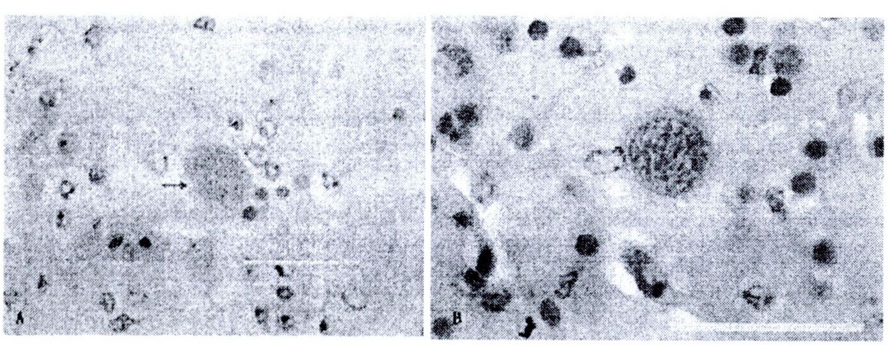

Figure 5 *N. caninum* cyst (arrow head) in the brain of a bovine aborted foetus. (A) Hematoxylin and eosin staining. (B) Immunohistochemical staining with canine anti-*N. caninum* antibodies. Bar = 50 mm (Zhang et al., 2007).

7.3 Polymerase chain reaction

The PCR has contributed a key role to the neosporosis diagnosis for its high sensitivity and specificity. Brain and body tissue samples were used most commonly

(Suteu et al., 2010; Wang et al., 2009; Yao et al., 2009). Moreover, several other sample types have been used to successfully illustrate *N. caninum* DNA, i.e. amniotic fluid (Gottstein et al., 1999), cerebrospinal fluid (Buxton et al., 2001; Peters et al., 2000; Schatzberg et al., 2003), and infected dogs and coyotes' feces (Gondim et al., 2004b; Schares et al., 2005). Recently, *N. caninum* DNA was found in the blood, semen and milk of the cattle (Caetano-da-Silva et al., 2004; Ferre et al., 2005; Moskwa et al., 2007; Okeoma et al., 2004a, 2005; Ortega-Mora et al., 2003).

This method is both highly sensitive and specific but may be affected by certain factors including the autolysis of the samples, the distribution of parasites in the samples, appropriate primers, methods of template DNA extraction, PCR cycles and analysis of the results (Dubey and Schares, 2006; Jenkins et al., 2002). To increase the sensitivity of the test, nested-PCR with primary and secondary multiplications was chosen (Barratt et al., 2008; Cabral et al., 2009; Medina et al., 2006; Yao et al., 2009). Furthermore, number of tachyzoites in semen of the bulls, blood of infected mice and bovine aborted fetuses was quantified by quantitative PCRs (Caetano-da-Silva et al., 2004; Collantes-Fernandez et al., 2002; Ferre et al., 2005; Ortega-Mora et al., 2003; Pinitkiatisakul et al., 2008). In addition, distinguishing *N. caninum* strains was carried out by the use of multiplex –PCR (Al-Qassab et al., 2009, 2010). The target genes used to establish the diagnosis of *N. caninum* were 18S rDNA, 28S rDNA, internal transcribed spacer 1 (ITS1) and pNc5 gene (Bartley et al., 2006; Cheah et al., 2004; Ellis, 1998; Okeoma et al., 2004a; Pedraza-Diaz et al., 2009; Pena et al., 2007; Sanchez et al., 2009) (Figure 6).

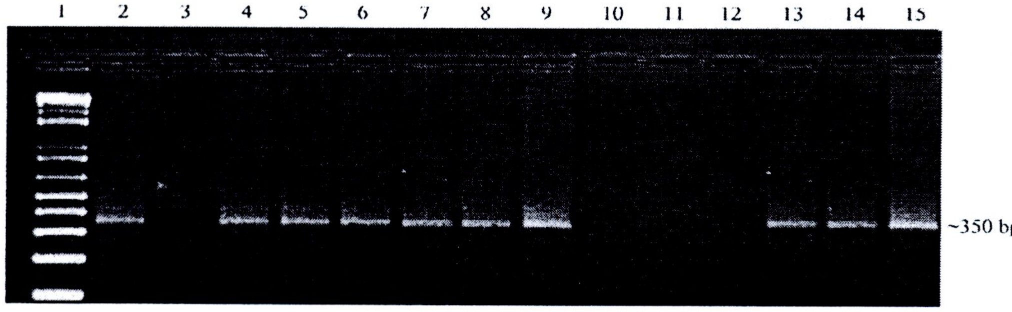

Figure 6 PCR product of Nc-5 fragment amplified with primer pair Np21+ and Np6+. Lane 1:1kb ladder, lanes 2 and 3: positive and negative controls, respectively,

lanes 4-9: products from sero-positive heifers after abortion, lanes 10-12: products from sero-negative pregnant heifer, lanes 13-15: products from sero positive pregnant heifers (Okeoma et al., 2004a).

7.4 Serological methods

Test of presence of antibodies against *N. caninum* is, perhaps, the most popular method to diagnose neosporosis (Dubey et al., 2007a). Antibody assays including indirect fluorescent antibody test (IFAT), enzyme linked immunosorbent assays (ELISA), direct agglutination test (DAT), western blot and Rapid immuno-chromatographic test have been developed.

7.4.1 Enzyme linked immunosorbent assays

Enzyme linked immunosorbent assay tests were a preferable serological tool since it is not time-consuming and a large number of samples can be analyzed at the same moment. This method can be utilized to demonstrate the *N. caninum* specific antibodies in fetuses' fluid, blood, individual or bulk tank milk (Bjorkman et al., 1997; Frossling et al., 2006; Osawa et al., 1998; Yao et al., 2009). Whole tachyzoites lysate, whole fixed tachyzoites, recombinant protein, iscom incorporated tachyzoites have been used as the antigens in the ELISAs.

ELISAs have been used to diagnose neosporosis with different purposes. Serological examinations on herd level can be conducted by this technique (Banales et al., 2006; Chanlun et al., 2006a, 2006b; Garcia-Vazquez et al., 2009; Holmdahl et al., 1997; Rinaldi et al., 2005). ELISAs can also be applied to study the serological status of the aborted fetuses (Yao et al., 2009). However, the sensitivity of the test in this circumstance may be affected by immuno-competence of the fetuses which is complete at about 100-150 days of gestation (Osburn et al., 1982). Transplacental transmission was also investigated by using ELISAs (Bartels et al., 2007a; Bergeron et al., 2000; Davison et al., 1999; Schares et al., 1998) based on the fact that specific antibodies could not transfer from mother to calves during pregnancies, so the presence of *N. caninum* antibodies in the pre-colostrum calves could infer to the vertical transmission. Studying association between abortion and seropositivity to *N. caninum* was also carried out by using ELISA (Weston et al., 2005). The seropositive animals usually had higher rate of abortion than those were negative (Lista-Alves et al., 2006; Lopez-Gatius et al., 2004a; Oshiro et al., 2007). To distinguish chronic infection from a recent infection in cattle, avidity ELISA was developed (Bjorkman et al., 1999). This method based on the principle that affinity of the antibodies produced in an acute infection to antigens is lower than that of antibodies produced in a chronic infection to antigens. Avidity ELISA can also be a valuable tool to study the duration of infection (Björkman et al., 2003, 2005; Jenkins et al., 2000; Liao et al., 2005; McAllister et al., 2000). Recently, another recombinant protein-based ELISA has been used to differentiate primo-infection, re-infection, recrudescence and chronic infection (Aguado-Martinez et al., 2008). The test used both antigens of tachyzoites and bradyzoites. The presence of antibodies against tachyzoite antigens indicated a primo-infection, re-infection or recrudescence while presence of antibodies against bradyzoite antigens inferring a chronic infection. In addition, an iscom ELISA with the sensitivity and specificity of 99% and 96%, respectively, has been applied in diagnosis of neosporosis in cattle and dogs (Björkman et al., 1994; Chanlun et al., 2007; Frössling et al., 2003)

7.4.2 Indirect fluorescent antibody test (IFAT)

The IFAT is the first test used to diagnose neosporosis in dogs (Dubey et al., 1998). The principle of the test bases on the detection of antibodies directed to antigens on the cell surface of the tachyzoite; such antigens, in the Apicomplexa are more specific than intracellular ones (Bjorkman and Uggla, 1999). The *N. caninum* IFAT has been applied to detect antibodies from a large number of animals, including dogs, foxes, cats, cattle, sheep, goats, horses, rodents, primates and water buffaloes (Bjorkman and Uggla, 1999). This test was generally used as a reference test (Bjorkman and Uggla, 1999; Dubey et al., 1996; Hemphill et al., 2000). However, the performance of this test depends on visual and individual interpretation, so the results may be a certain degree subjective (Bjorkman and Uggla, 1999). Moreover, no generally accepted cut-off titer was established for this test which considerably varies, i.e. 1:20 (Sedlak and Bartova, 2006), 1:25 (Fujii et al., 2001; Gennari et al., 2005), 1:50 (Faria et al., 2010; Ueno et al., 2009), 1:100 (Campero et al., 2007; Rodrigues et al., 2005) and 1:640 (Huong et al., 1998). The sensitivity of an IFAT was also judged to be lower than expected which suggested that the test could not be used as a true gold standard test (Frossling et al., 2003).

7.4.3 Direct agglutination test (DAT)

The DAT is a highly sensitive and specific for testing both experimentally and naturally infected animals, highly reproducible between and within readers, easy to use on large sample sizes without requirement of special equipments, and useful in testing serum from any species without modification (Packham et al., 1998). This test enrolled either formalin persevered whole tachyzoites or mouse-derived tachyzoites as the antigens (Dubey et al., 1998; Gennari et al., 2002). The DAT was used to investigate the seroprevalence of a very large type of animals including buffaloes (Fujii et al., 2001), cattle (Canada et al., 2004a), horses (Pitel et al., 2001), camels (Hilali et al., 1998), white-tailed deer (Dubey et al., 1999), black-tailed deer (Dubey et al., 2008), cats (Dubey et al., 2002b), dogs (Ferroglio et al., 2007b), foxes (Jakubek et al., 2001), raccoons (Lindsay et al., 2001b), gerbils (Basso et al., 2001a), brown hare (Ezio and Anna, 2003), several wild animals (Dubey and Thulliez, 2005) and marine mammals (Dubey, 2003). Moreover, this serological test was also applied to assess the effects of serologic status of *N. caninum* on weight gain and feed efficiency in post-weaning beef steers (Barling et al., 2000, 2001), on an outbreak of *Neospora caninum* - associated abortion (Jenkins et al., 2000). It was also used to evaluate the efficacy of a *N. caninum* tachyzoites vaccine in protection against fetal loss in experimentally infected pregnant ewes (Jenkins et al., 2004).

7.4.4 Immunoblot and Rapid immuno-chromatographic test

Immuno blot was first used to analyze the IgG response of sheep, goat and cattle infected with *N. caninum* (Harkins et al., 1998). The efficiency of transmissions of *N. caninum* in dairy cattle, dogs and foxes was also investigated by using this test combined with IFAT and ELISA (Schares et al., 1999, 2001a, 2001b). This serological test was found to improve the foetal serology in bovine neosporosis (Sondgen et al., 2001) and to be superior to ELISA when using degraded blood samples collected from deer carcasses (Anderson T et al., 2007). Also, an avidity immunoblot could differentiate between acute and chronic neosporosis in cattle (Aguado-Martinez et al., 2005). Moreover, immuno blot was applied to interpret the infection stage of bovine neosporosis by recognition of different antigenic proteins of *N. caninum* by bovine IgA, IgE, IgG, IgM (Shin et al., 2005). Those authors suggested that the comparison of immunoblot profiles with different immunoglobulin classes might be useful for strategies to develop effective vaccine candidates against neosporosis. In experimentally infected dogs, immunoblot could detect antibody reactivity at 1 to 3 days earlier than those found in ELISA (Staubli et al., 2006). In general, immunoblot is a very sensitive and specific complementary tool to improve the serology for *N. caninum* infection in cattle (Staubli et al., 2006).

Another serological method was used to diagnose neosporosis was rapid immuno-chromatographic test. This approach had a very good agreement with ELISA and was stated to be simple, rapid, sensitive and specific for the detection of antibodies to *N. caninum* in cattle and suitable for the clinical or field application (Liao et al., 2005). The authors also referred to the ability to detect all classes of immunoglobulins of the test. However, in that study, only 24 samples collected from four mice and four dogs were analyzed, that aroused a need for further evaluation of the performance and application of this test.

8. Economic losses

The economic loss due to *N. caninum* has been reported in only cattle despite the facts that neosporosis is also available in several other domestic and wild animals. The direct damage is fetal loss beside the indirect loss including cost of reduced milk production, culling and replacement, low weigh gain, veterinary cost, rebreeding and diagnosis.

Abortion is the most significant loss caused by neosporosis (Anderson M.L et al., 1991; Hernandez et al., 2002; Kul et al., 2009; Pabon et al., 2007). Seropositive cows might have up to 22.3 times higher risk of abortion than seronegative cows (Lopez-Gatius et al., 2004a; Weston et al., 2005). The loss of from 13% to 44% pregnancies in positive cattle were reported commonly (Dijkstra et al., 2001a, 2002; Lopez-Gatiuz et al., 2004a; McAllister et al., 2000; Schares et al., 2002a).

Milk production and milk quality in the positive cattle were lower than those in their negative counterparts. A lower milk and fat production of 3.1 lb/cow/day and 0.14 lb/cow/day was reported by (Thurmond and Hietala, 1997b). Hernandez et al. (2001) found that each positive cow produced 3-4% milk less than negative cow and the cost due to neosporosis was $128 /cow/lactation. Milk, fat and protein yield were declined 158 kg, 5.5 kg and 3.3 kg each lactation, respectively (Tiwari et al., 2007). Another study also found an association between serostatus and reduced milk production in aborted herds (Hobson et al., 2002).

Neosporosis can cause economic loss due to the increase in number of services per conception in positive cows compared with that in their negative cows (Hall et al., 2005). Also, in that study day open had a trend to be longer in the seropositive cows than their negative counterparts. Chances of a positive heifer not to conceive was 1.8 times higher than those of negative heifers (Munoz-Zanzi et al., 2004).

The risk of being culled was also higher, i.e 1.6 times to 1.9 times in the positive compared with the negative cattle (Bartels et al., 2006b; Thurmond and Hietala, 1996; Tiwari et al., 2005; Waldner et al., 1998). In the herd with high serostatus, the culling risk was 1.73 times higher than the herd with low serostatus and free of neosporosis (Bartels et al., 2006b). In beef cattle, neosporosis resulted in reduced post-weaning weigh. In each seropositive calf, owners lost $ 15.62 (Barling et al., 2000).

In Switzerland, the annual loss in dairy industry induced by neosporosis was estimated to be 9.7 euros in total. In detail, farmer lost 1.9-2,0 millions euros, 123.000-160.000 euros, 5.9 million euros and 1.6 millions euros for the loss due to abortion, cost of veterinary service, loss of reduced milk yield and premature culling, respectively (Hasler et al., 2006a). In California where there were about 40,000 abortions due to neosporosis annually, *N. caninum* was measured to cause $ 35 million (Barr, 1998). In Australia and New Zealand the loss because of neosporosis was considered up to 100 million Australian dollars per year (Reichel, 2000). Each 50-dairy cow herd in Canada lost 2,304 euros every year (Chi et al., 2002). In Netherlands, 76% seropositive farms without abortions did not endure the loss due to neosporosis. By contrast, 24% remaining farms in which the abortions occurred might have lost up to 2,000 euros/farm/per year (Barling et al., 2000).

The loss predisposed by neosporosis in the cattle industry is really substantially significant. *N. caninum* has been reported worldwide but the economic damage has been estimated in only a few countries. It should be born in mind that the real loss caused by neosporosis in the animal production should be much higher than those have been demonstrated.

9. Prevention and control of neosporosis

Prevention and control of neosporosis based on the reduction of number of positive animals in the herds by decreasing the risk of both vertical and horizontal transmission. Quite several approaches have been proposed including "testing and culling", improvement of the bio-security of the farms, reproductive management, chemotherapy and vaccination.

Plenty of efforts have been made to reduce the prevalence of neosporosis in herds by reducing the vertical transmission. Testing the whole herd and culling all the positive animals were considered the most effective measure to eradicate neosporosis (Hall et al., 2005; Hasler et al., 2006b; Reichel and Ellis, 2006). However, this solution was still criticized for its economic impacts, and this might result in the change of gene system, structure of the herds and its effects on the stabilization of the meet market (Hasler et al., 2006a; Larson et al., 2004). Similarly, two controls as culling female that fail to give birth to a calf and selling seropositive female cattle and purchasing seronegative replacement female cattle were not likely to be economically beneficial. Alternatively, the policy of discontinuing breeding the offspring of the positive dams seemed to be the suitable choice for its advantages in the aspect of economics though the efficiency was lower than the former measures (Larson et al., 2004).

Neosporosis will not be able to survive if there was no horizontal transmission between definitive and intermediate hosts. Presence of dogs in farms positively associated with the prevalence of the infection (Corbellini et al., 2006; Otranto et al., 2003; Schares et al., 2004), seroconversion of the cattle (Dijkstra et al., 2002) and storm abortion within herds (Bartels et al., 1999; McAllister et al., 1996a, 2000). With those findings, it is sensible and plausible to restrict contact between dogs and cattle to reduce the transmission and prevalence of the infection as well. The aborted fetuses and placenta, infected tissues from calves and cows should not be within the access of the dogs. Food and water provided to cattle should be covered and protected from the infection of oocysts. Since several rodents such as mice, rats and rabbits were infected with *N. caninum* (Ferroglio et al., 2007a; Hughes et al., 2008; Jenkins et al., 2007; Romano et al., 2009) farms of animals should be free of these rodents so that definitive hosts will not get infected by eating them and transmit disease to the cattle. A similar policy should be applied to poultry since chickens and pigeons are possible intermediate hosts of the parasite (Costa et al., 2008; Mineo et al., 2009).

Some reproductive resolutions have been suggested to prevent and control neosporosis. Use of beef bull semen to inseminate dairy cows could reduce the risk of abortion (Lopez-Gatius et al., 2005). However, in the aspect of epidemiology this is not a prudent choice because it can not reduce the transmission. Based on the fact that early cattle embryos did not expose to the parasites (Moskwa et al., 2008), embryo transfer using the positive donors and negative receivers could be a better option (Baillargeon et al., 2001; Campero et al., 2003; Landmann et al., 2002).

Currently, little information about chemotherapy for treatment of neosporosis is available. Some drugs such as toltrazuril and its derivative named ponazuril, and thiazolide were experimentally used *in vitro* (Esposito et al., 2005, 2007a, 2007b; Muller et al., 2008). In mice, toltrazuril was found to reduce fetal losses and diaplacental passage of the parasites to the fetal brain (Gottstein et al., 2005). Those authors also reported that toltrazuril and ponazuril could completely prevent the formation of cerebral lesions in the experimentally infected mice (Gottstein et al., 2001). Toltrazuril could also increase the rate of survival of congenitally infected mice (Strohbusch et al., 2009). Haerdi et al. (2006) demonstrated that toltrazuril had the potential to eliminate *N. caninum* in newborn calves. In other studies, ponazuril was able to protect experimental *N. caninum* infection calves (Kritzner et al., 2002).

Protection of animals from neosporosis by vaccines is still facing difficulties since there are no highly efficacious vaccines were proven. Several types of vaccines have been studied their efficacy including live tachyzoite, killed whole tachyzoite, whole tachyzoite lysate, gamma irradiated tachyzoite, tachyzoite surface protein and recombinant protein vaccines (Andrianarivo et al., 1999; Haldorson et al., 2005; Heuer, 2003; Jenkins et al., 2004; Ramamoorthy et al., 2007a, 2007b; Romero et al., 2004; Williams D.J et al., 2007). Live tachyzoite vaccine was found to confer protection against fetal death in cattle while whole lysate tachyzoite vaccine failed (Williams D.J et al., 2007). Recombinant vaccines have shown controversial effects on prevention of infection in mice (Aguado-Martinez et al., 2009; Debache et al., 2009; Ellis et al., 2008). Using surface protein as a vaccine candidate, Zhao et al. (2009) and Haldorson et al. (2005) obtained controversial results. A commercial killed whole tachyzoite vaccine - Bovilis Neoguard - failed to confer a stable efficacy of protecting cattle from abortion since its efficiency ranged from 0 % to 54% (Heuer, 2003; Romero et al., 2004). Another killed vaccine succeeded in improving fetal survival but failed to reduce congenital infection in sheep (Jenkins et al., 2004). In a study using gamma irradiated tachyzoite as the vaccine, all the vaccinated mice were healthy and survived after day 25 post-challenge while the whole group of unvaccinated mice died within a week (Ramamoorthy et al., 2006).

10. Neosporosis in Thailand

Antibodies to *N. caninum* were first demonstrated in central Thailand using IFAT in which the prevalence in dairy cattle was reported at 6% (46/904) (Suteeraparp et al., 1999). These results were in agreement with those found in 549 dairy cows in Nakhon Pathom which was 5.5% (Kyaw et al., 2004). In the former study, though the infection status in 11 central provinces was fairly low, prevalences in 2 provinces in Northeast were, in contrast, much higher ranged from 12% to 44%. The high prevalence of infection in Northeast was explained by the new introduction of the animals to these areas and the stressful pregnant conditions were advantageous to the parasites to infect the cattle. Newly introduced condition was also suggested as one of reason that caused high prevalence of *N. caninum* infection in dairy cattle in Northeast (Kashiwazaki et al., 2001). Prevalences of antibodies against *N. caninum* in dairy cattle herds with history of abortion in Muang and Nongbua Lamphu were fast continuously increased within a year from the time of introduction of the animals, i.e. from 37,5% to 62.5% and from 50 to 70%, respectively (Kashiwazaki et al., 2001). Similarly, in aborted dairy and beef cattle from six provinces in Northeast, the prevalence of *N. caninum* infection was 40,2% and 61.8%, respectively (Charoenchai et al., 2000).

The effects of dogs on the infection of the parasite in dairy cattle were not detected in Thailand. This may be due to the low proportion of positive dogs, i.e. 1.2% (Kyaw et al., 2004). Similarly, herd prevalence can keep a negative infection status despite the frequent presence of the dogs (Chanlun et al., 2006b). This was in line with the finding that there was no relationship between age and infection of the cows. However, herd size did affect the infection status in which the bigger herds had higher prevalences than the smaller herds (Kyaw et al., 2004).

In a long-term study on 11 dairy herds in Khon Kaen during the years of 2001-2004, prevalence of antibodies against *N. caninum* was fluctuated within 10-13% while the vertical and horizontal transmission were 58% and 5%, respectively (Chanlun et al., 2007). Also, the first congenital transmission of *N. caninum* in dairy cattle was firstly reported in Thailand (Kyaw et al., 2005). Beside sera samples, bulk tank milk and individual milk were also used as alternative samples to study neosporosis in dairy cattle (Chanlun et al., 2002, 2006a, 2006b)

11. Buffalo raising in Thailand

In Thailand, most of buffaloes are classified as swamp type. During period of 1971-1981, there were 5.5-6.5 millions swamp buffaloes leading the country to be one of the countries possessing largest buffalo's populations in the world. However, the number of buffaloes in Thailand has been experienced a dramatical decline. In the advent of the tractors, the role of swamp buffaloes as the "draught machine" has been shifted to the real machines - the tractors, and farmers became to pay less attention to their buffaloes.

Moreover, the introduction of industries to the agricultural areas has occupied the habitats of the swamp buffaloes. In addition, that a job at the industrial zone is paid higher than rearing the swamp buffalo has brought the farmers out of the villages which resulted in lacking of people who took care of, and were interested in swamp buffaloes. Consequently, buffalo population had reduced from 4.4 million heads in 1985, to 1.8 million head in 1999. During 2000-2008 the annual average decline rate had been 7.6%. According to the statistics by Department of Livestock Development, Thailand, in 2009, there were 1.39 million buffaloes being distributed widely with the majority in North-Eastern area which accounted for 74% of the whole country's buffalo population (DLD, 2008, 2009) (Figure 7). In this area, buffaloes were mostly raised in small farms less than 20 heads in each. Only 4.5 % buffaloes were kept in farms having more than 20 heads (DLD, 2004). Totally, approximate 301,000 householders involved in the buffalo raising in Thailand (DLD, 2009).

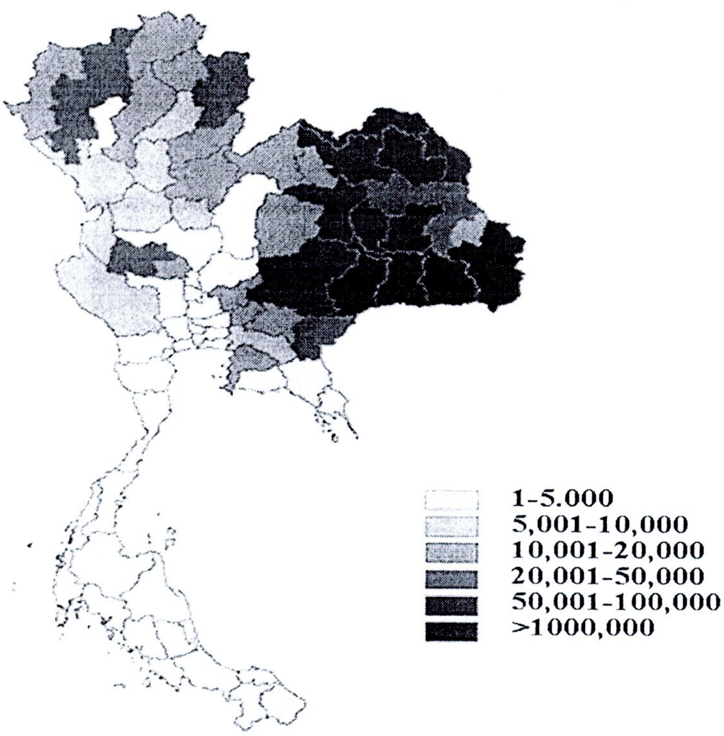

Figure 7 Swamp buffaloes population map in Thailand (modified from DLD, 2005)

Beside natural mating which has been popular breeding program in swamp buffaloes, there are several reports of using hormones and artificial insemination to improve the reproductive performance in this species in Thailand. A very promising conception rate of 68.2% was obtained when (Estrumate) and AI were applied (Kamonpatana et al., 1982). Different doses of (Lutalyse) confered various results. Using 10mg, 15mg, 20mg and 25mg Lutalyse per injection, those authors reported that the conception rates were 33.3%, 37.5%, 40.9% and 50,0%, respectively (Kamonpatana et al., 1984b). However, the results were not stable, the application of 25mg per injection resulted in 21.2% of pregnancy rate (Kamonpatana et al., 1984a). Other ovsynch utilizing $PGF_{2\alpha}$ has achieved quite a high fertility rates of about 40% (Bodhipaksha et al., 1984). In the attempt to induce oestrus of buffalo cows and heifers, CIDR-B gave a higher conception rate in buffalo cows of 30.0% than that of 23,3% in buffalo heifers (Utha et al., 2002). Recently, GnRH and $PGF_{2\alpha}$ were employed concurrently in an ovsynch that gained a conception rate of 34.9% which was not different from the pregnancy rate in the natural oestrus swamp buffaloes, i.e. 34.6% (Chaikhun et al., 2009). The efficiency of ovsynch programs and artificial insemination is required to be ameliorated, and the use of artificial insemination in swamp buffaloes in Thailand is still restricted.

Though the swamp buffalo population in Thailand has been decreased, its role in the economy of Thailand, daily life of Thai people, especially Thai farmers is still important. For the farmers, they can raise buffaloes for several purposes. Buffaloes can eat and digest agricultural sub-products and grass to survive. Farmer may not need to feed them concentrate as they do with dairy cattle. Therefore, the raising of swamp buffaloes as a "saving bank" gives some financial security for the farmers (Indramangala, 2001). Furthermore, buffaloes can work as draught machines which do not need oiling and repairing. In addition, the manure produced by buffaloes can be beneficially used for crops instead of chemical fertilizer. Not contributing to only the living of Thai people, swamp buffaloes also play a role in the economy of Thailand. Each year, about 87,000 swamp buffaloes can be sent to slaughterhouse for meat and hides consumption (DLD, 2007). Although swamp buffaloes do not help much in exportation, they serve the demands of the country, and reduce the amount of buffaloes products such as hides which Thailand has to import (DLD, 2006).