CHAPTER V
DISCUSSION

1. Investigation of prevalence of *N. caninum* infection in swamp buffaloes in five provinces in Northeast Thailand

In this study, antibodies to *N. caninum* in swamp buffaloes and beef cattle were tested by Iscom ELISA which was used to study the infection of this parasite in swamp buffaloes and dairy cattle (Bjorkman et al., 1997; Huong et al., 1998). Validation of Iscom-ELISA to detect antibodies to *N. caninum* in dairy cattle conferred 99% sensitivity and 96% specificity (Frossling et al., 2003). Furthermore, the use of anti-bovine antibody conjugate in ELISA (Mohamad et al., 2007; Yu et al., 2007) and IFAT (Campero et al., 2007; Fujii et al., 2001; Gennari et al., 2005; Rodrigues et al., 2005) to demonstrate the antibodies to *N. caninum* in buffaloes were widely reported.

In a total of 532 swamp buffaloes investigated, 24 of them were seropositive with *N. caninum* accounting for 4.5%. These results were in line with those previously reported in other neighboring countries. In Vietnam, 1.5% positive swamp buffaloes were demonstrated (Huong et al., 1998). Similarly, a study from the Philippines also showed a low prevalence of *N. caninum* infection which was 2% in their swamp buffaloes (Konnai et al., 2008). In addition, Yu et al. (2007) did not find any infected animals out of 40 surveyed swamp buffaloes in China.

On the contrary, several studies in river buffaloes found very high seroprevalence of *N. caninum*. In a large scale investigation in Italy, 34% river buffaloes were infected with the parasite (Guarino et al., 2000). A similar result was also found in Iran where 37% river buffaloes were positive (Mohamad et al., 2007). More severely, the infection status was even much higher in Argentina and Brazil where 64% to 71% river buffaloes were detected positive with *N. caninum* (Campero et al., 2007; Fujii et al., 2001; Gennari et al., 2005).

Interestingly, results of this study agreed with those reported in swamp buffaloes and disagreed with those found in river buffaloes. Prevalence of infection found in river buffaloes was high in many studies using anti-bovine conjugate. This indicated that anti-bovine conjugate can be applied to swamp buffalo samples, and was not the factor that led to the low prevalence in swamp buffaloes. Except the study conducted in Italy (Guarino et al., 2000) which used anti-buffalo conjugate in the test, other studies used anti-bovine conjugates (Campero et al., 2007; Fujii et al., 2001; Gennari et al., 2005; Rodrigues et al., 2005). It is also ruled out the notion that the difference in the test performance between ELISAs and IFATs may predispose largely different results. Both ELISAs (Meenakshi et al., 2007; Mohamad et al., 2007) and IFATs (Campero et al., 2007; Fujii et al., 2001; Gennari et al., 2005; Rodrigues et al., 2005) have conferred high prevalence of antibodies against *N. caninum* in river buffaloes such as 37 to 50% and 34% to 70.9%, respectively. Furthermore, the agreement between an Iscom ELISA and IFAT which was used a titre of 1:25 to test neosporosis in cows was good (Jenkins et al., 2000). In this study, the low exposure to the parasite is unlikely to be a suitable explanation for the low prevalence of infection in the swamp buffaloes because in Khon Kaen beef cattle had a significantly higher chance of being positive compared with swamp buffaloes though they lived in the same area. Therefore, the low *N. caninum* seroprevalence in swamp buffaloes in the present study may be due, to some extent, to the resistance of the swamp buffaloes to *N. caninum*.

Swamp buffaloes are highly resistant to *Oesophogostomum radiatum* though that parasite can cause heavy infection resulting in weight loss and sometimes death in Italian river buffaloes (Frisch and Vercoe, 1979). Furthermore, blood parasitic diseases such as Anaplasmosis, Babesiosis and Trypanosomiasis were more heavily infected in Murrah buffaloes than those in swamp buffaloes in southern provinces of Vietnam (Hung, 1985). A study of mitochondrial DNA of buffaloes suggested that river and swamp buffaloes were two different clades and domesticated independently (Kumar et al., 2007). The difference in Th1 and Th2 cytokines in these two subtypes of buffaloes was also reported (Mingala et al., 2006, 2007). In those studies, they found that there were substitutions of amino acid in TNF- α, IFN-γ, IL-1α, IL-4, IL-10 and IL-12 of swamp buffaloes compared to those of river buffaloes. IFN-γ, IL-12 and TNF- α have been proven to play an important role in host immunity to *N. caninum* and inhibition of the parasite multiplication (Baszler et al., 1999; Innes et al., 1995; Khan et al., 1997; Yamane et al., 2000). Moreover, the alteration of a single amino acid in IL-10 could lead to the change of the immunostimulatory activity in a *in vitro* experiment (Ding et al., 2000). Other studies have also obtained similar results when an alteration of a single amino acid in a cytokine made the change to the immunological response (Blander et al., 2000; Harada et al., 2003). Therefore, the incomplete homology of amino acid sequence in those cytokines between river and swamp buffaloes may result in the different immune response of these two subtypes of buffaloes to *N. caninum* which could lead to the dissimilarity in the prevalence of *N. caninum* infection. Besides, feeding, managements, concentration of buffaloes could be others possible fatocts influencing the prevalence of *N. caninum* infection in swamp and river buffaloes.

In the present study, the prevalence of the *N. caninum* infection in swamp buffaloes ranged from 0% to 6.4% in 5 provinces which was insignificant different. In Nong Khai province, none of buffaloes were found positive out of 31 investigated animals. It was not able to conclude that there was no infection in this province but it suggested that the prevalence would have been low if more samples had been collected. Studying at 4 ranches in one province in Argentina, Campero et al. (2007) found that the proportion of positive river buffaloes varied between locations. Gennari et al. (2005) obtained a similar result when the *N. caninum* infection status at three buffalo farms in Pará state from Brazil was different from one another.

Prevalence of *N. caninum* infection in female and male buffaloes were 6.8% and 5.1%, respectively. Gender of the buffaloes in this study did not affect the probability of being infected with *N. caninum*. This agreed with the finding by Campero et al. (2007). By contrast, that female buffaloes were likely to be more susceptible to *N. caninum* than the male was supported by Mohamad et al. (2007). In that study, 43.3% female buffaloes were positive while only 15% male were infected.

Age was considered a risk factor of the neosporosis in buffaloes. Fujii et al.

(2001) reported that buffaloes at 6 to 8 years old were at the highest chance of positivity. Buffalo cows were also more likely to be positive than buffalo heifers (Mohamad et al., 2007). An increasing prevalence when the buffaloes got older was also demonstrated by Guarino et al. (2000). In that study, the prevalence of infection increased from 24.5% when buffaloes were at 1 to 2 years old to 43.1% when buffaloes were six years old or older. All of those findings suggested the existence of horizontal transmission in water buffaloes. In present study, the buffaloes older than 3.5 years old were at significantly higher risk of being positive than the younger ones. The explanation for the finding was that the accumulative infection in the older buffaloes resulted in the higher prevalence in this group than the younger group.

Also, it is quite interesting to find that out of 88 buffaloes under 2 years old, none of animals were positive with *N. caninum*. Furthermore, in 5 pairs of dams-calves (in both collections, 3 pairs in the first and 2 pairs in the second), all of the 5 dams were positive while their 5 calves were negative. The result suggested that all of these 5 calves might be born healthily without transplacental transmission. Similar observation was reported in dairy cattle in Thailand. Three of the four calves born to the infected dams were negative (Kyaw et al., 205). This opposed the result in a study on buffaloes in Brazil in which the authors reported that transplacental transmission could be up to 73.9% (Rodrigues et al., 2005). The lack of positive buffaloes those were under 2 years old suggested that the horizontal transmission in swamp buffaloes was essential to remain the infection within the herd. The postnatal spreading of the infection emphasizes the important role of the dogs which have been known to increase the risk of infection in cattle (Bartels et al., 2007b; Gonzalez-Warleta et al., 2008).

In the present study, there was a trend that buffaloes at 3.5 to 4.5 years old were at higher risk of being positive than others. The reasons why buffaloes 3.5 to 4.5 years old were more likely to be positive than those older than 4.5 years old were unknown. The mean age at puberty of swamp buffaloes was reported to be at about 3 years old (Gordon, 1996). After animals reach puberty, there are the changes in the levels of seveval hormones such as progesterone, estrogen in the female, and testosterone in the male.

These hormones have been reviewed to have effects on the immunity of the host by increasing or decreasing certain of many of preimflamatory cytokines which play their roles in the control of the parasite (Robert et al., 1996). Furthermore, during gestation, cell mediated immunity is also modulated to facilitate the pregnancy. Innes et al. (2001) indicated that there is a significant change in the maternal immune response around mid-gestation compared to that at early gestation, and that at this time the mother may be less able to cope with the *N. caninum* which in a persistently infected animal may lead to recrudescence of infection. This could be one of the reasons that predisposed the high risk of being positive in the buffaloes 3.5 to 4.5 years old.

Abortion risk and vertical transmission rate in cattle have been found to reduce in the subsequent pregnancies (McCann et al., 2007; Thurmond and Hietala et al., 1997). Those results suggested that the severity of *N. caninum* infection may decrease over time. After getting infection, the antibody titer could be fluctuated. Rodrigues et al. (2005) inoculated 6 buffaloes with *N. caninum*, all of the six buffaloes developed the antibody titers of 1:400 to 1:1600 in IFAT, usually after 3 weeks post inoculation. However, after 12 months, the antibody titers of all the buffaloes reduced to 1:25 to 1:50. There was also evidence of seroconversion of *N. caninum* infection in the cattle. In an interval of 3 to 12 months, 212 (39%) out of 543 initially positive cattle became seronegative in the second serum samples (Sager et al., 2001). Similarly, the fluctuation in the levels of specific antibody throughout a yearlong study was also reported (Maley et al., 2001). Moreover, in a study on seroconversion of *N. caninum* in dairy cattle in Thailand show that all of the four cows those were positive pre-breeding with an IFAT at the antibody titers of 1:400 to 1:1600 became seronegative at parturition at the antibody titer of 1:200 (Kyaw et al., 2005). Those results suggested that there could be fluctuation of antibodies to *N. caninum* in swamp buffaloes older than 4.5 years old and resulted in the finding that buffaloes older than 4.5 years old were more likely to be at lower risk of being positive than those at 3.5 to 4.5 years old.

2. Study of effects of *N. caninum* infection on the fertility of artificially inseminated swamp buffaloes

The effects of *N. caninum* infection on the reproductive parameter such as abortion in both beef and dairy cattle were widely reported. Seropositive cattle had higher risk of being aborted than their negative counterpart (Koiwai et al., 2005b; Weston et al., 2005). *N. caninum* may be attributable to from 21% to 26% pregnancy loss in cattle (Garcia-Vazquez et al., 2005; Koiwai et al., 2005a). In some herds, the abortion rates in positive cattle can be up to 44% (Lopez-Gatius et al., 2004a). In buffaloes, though some abortion cases were suspected to be due to *N. caninum* (Guarino et al., 2000), the effects of the parasite on the reproductive parameters were lacking.

The present study was to investigate whether *N. caninum* infection affected the conception rate of swamp buffaloes at day 45th to 60th post artificial insemination. The conception rate of swamp buffaloes in this study was low. Only 10.3% (22/213) buffaloes were pregnant (data not shown). Unfortunately, many buffaloes which had serological records did not have pregnancy records and vice versa. As the results, only 115 buffaloes could be included in the study. From those buffaloes, the overall pregnancy rate was 9.6% (11/115) while that of negative and positive buffaloes was 9.2% (10/109) and 16.7% (1/6), respectively. The conception rate in Thai swamp buffaloes were found various in several studies. Fertility rates in artificially inseminated buffaloes ranging from 30% to 40% were widely observed (Bodhipaksha et al., 1984; Chaikhun et al., 2009; Kamonpatana et al., 1984b; Utha et al., 2002). Interestingly, a fertility rate of 68.2% was obtained by Kamonpatana et al. (1982). However, the same authors also showed a humble conception rate which was 21.2% (Kamonpatana et al., 1984a). Low conception rate in this study may be due to the fact that all the female buffaloes those were led to the site of work had been enrolled into the synchronized programs. Poor selection of the swamp buffaloes may lead to the utilization of sterile animals which resulted in low conception rate. Moreover, the efficiency of the hormone programs using $PGF_{2\alpha}$ or GnRH depended on the presence of a corpus luteum or a dominant follicle at the time of treatment, respectively (De Rensis and López-Gatius et al., 2007). In the present study, buffaloes had not been checked for the existence of either a corpus luteum or a dominant follicle at the beginning of the program. This could be one of the reasons of the low conception rate of the swamp buffaloes in this study compared to those controlled the presence of a dominant follicle at the commencement of the treatment (Chaikhun et al., 2009). Furthermore, the time interval from the treatment of $PGF_{2\alpha}$ to the oestrus has been published to vary from 2-6 days (De Rensis and López-Gatius et al., 2007), and this variance also hampered the use of fixed-time AI. It is also necessary to be informed that it is very difficult to work with swamp buffaloes in Thailand. Most of buffaloes did not have any records of age, gestation, abortion and other parameters. Furthermore, because the animals were on the fields the whole day, so all the activities had to be carried out in the early morning before the farmers led their animals to the pasture. Due to the small number of investigated animals, the low conception rate and prevalence of infection, effects of *N. caninum* infection on the fertility rate of swamp buffaloes was not observed.

In this study, the abortion of buffaloes was not followed. In cattle, abortion usually occurs at 5th to 7th months of the gestation (Gonzalez et al., 1999; Kim et al., 2000; Moen et al., 1998; Thurmond et al., 1997). A longitudinal study of abortion and aborted fetuses may confer a better insight into the effects of *N. caninum* on the reproductive performance of the swamp buffaloes.

The influence of *N. caninum* infection on conception rate of cattle was controversial. *N. caninum* did not infect cattle embryos at day 7th of gestation in the positive dams (Landmann et al., 2002; Moskwa et al., 2008). In other project, the infection status was found to have no effects on the early pregnancy (34th to 90th days) of the cattle. Out of 183 abortions, 23 of them were positive accounting for 12.5%. Severely, during the day 90 to the end of gestation, there were 146 abortion in which 105 (71.9%) aborted dams were positive (Lopez-Gatius et al., 2004b). Lopez-Gatius et al. (2005a) stated that *N. caninum* infection did not affect the fertility of dairy cows in herds with high incidence of *Neospora*-associated abortion. In that study, the fertility rates of seropositive and seronegative dairy cows were 32.6% and 34,0%, respectively, without any significant difference. However, the abortion rate in positive cows was 30.1%

compared with that of 4.2% in the negative ones. In beef cattle, the pregnancy rate in seronegative animals had a trend to be higher than that in the seropositive counterparts though the difference was not significant (Bjorkman et al., 2003). Those authors also announced that *Neospora* infection did not have harmful consequence on the reproductive performance in a herd of dairy cattle (Bjorkman et al., 1996).

The calving interval, number of services per conception and pregnancy rate within 90 day post partum in dairy cattle were not affected by *N. caninum*. The number of services per conception in seropositive cattle even tended to be better than that in negative ones, i.e. 1.9 services/conception versus 2.3 services/conception (Jensen A.M et al., 1999). Similar results were also found (Bartels et al., 2006a). In that study, the calving interval, non-return rate, age at first calving and number of services per conception were not different between positive and negative cattle. Romero et al. (2005) showed that infection of *N. caninum* did not impact the number of services per conception. Other study did not find any significant influence of *N. caninum* on the reproductive performance of cattle (Canada et al., 2004a). A study in Thai dairy cattle reported that there was a trend that the positive cattle had longer calving interval compared with those were negative. However, it was not significantly different. Effects of *N. caninum* on other reproductive performance had not been found either (Chanlun et al., 2008).

On the other hand, some reports found the effects of *N. caninum* on some reproductive criteria on both negative and positive sides. First service conception in seropositive dairy cows was reduced (Vanleeuwen et al., 2010). Cattle that were positive in spring were more likely to be open in the fall (Waldner et al., 2001a). Also, *N. caninum* associated with an increased individual animal risk of non-pregnant and abortion (Waldner, 2005). An 1.7 times less likely to be pregnant in positive cows compared to negative cows was further uncovered (Waldner et al., 2001b). Similarly, negative beef cattle had twice as much chance as positive to be pregnant (Cunningham et al., 2000). Interestingly, positive aborted cows had higher chance (6.2 times) to be pregnant at the first artificial insemination after abortion than the negative aborted cows. The explanation for this observation was that the uterine involution process through the release of prostaglandin $F_{2\alpha}$ in positive aborted cows might be faster than that in negative aborted cows (Santolaria et al., 2009).

As the abortion usually occurs at second trimester to the end of gestation, the influence of the infection on the early pregnancy in animal was probably not serious. Furthermore, due to the small number of pregnant buffaloes and low prevalence of infection, effects of *N. caninum* infection on reproductive performance of swamp buffaloes were not detected. A longitudinal study to the full term of gestation may be necessary to examine the effects of the infection.

3. Prevalence of *N. caninum* infection in swamp buffaloes and beef cattle in Phu Wiang, Khon Kaen

There has been a steady growth of beef cattle population in Thailand. During the past ten years, the number of beef cattle has been increased from 4.9 millions to 8.6 millions. In the Northeast, proportion of beef cattle constantly accounts for more than 50% of the whole population in the country. There were 331,000 beef cattle in Khon Kaen province in 2009 (DLD, 2009).

In contrast with infection status in swamp buffaloes, prevalence of *N. caninum* antibodies in beef cattle in Khon Kaen was very high of 43.6%. In Thailand, neosporosis was first reported in dairy cattle in the central part (Suteeraparp et al., 1999). In the present study, the result was in line with that reported in Nongbua Lamphu and Loei provinces in which prevalence of infection was from 37.5% to 70% (Kashiwazaki et al., 2001). Similarly, in aborted dairy and beef cattle herds from six provinces in Northeast, the proportions of seropositive animals were 40.2% and 61.8%, respectively (Charoenchai et al., 2000). However, this was much higher than the finding that 10-13% dairy cattle in Khon Kaen was positive (Chanlun et al., 2007). Also, other studies in central part of Thailand found low frequencies of antibodies to *N. caninum* in dairy cattle, i.e. from 5.5% to 6% (Kyaw et al., 2004; Suteeraparp et al., 1999).

Comparing with reports from other countries, this may be the highest prevalence of *N. caninum* infection in beef cattle without history of abortion. Some studies showed quite a high positive proportion of beef cattle from 19% to 29.8% (Minervino et al., 2008;

Osawa et al., 2002; Sanderson et al., 2000). A very high prevalence of 79% was also detected in aborted beef cattle in United States (McAllister et al., 2000).

In the present study, effects of age of beef cattle on the *N. caninum* infection were not detected. However, there was a trend that beef cattle under 3 years old were less likely to be positive with *N. caninum* compared with those older. Whether age of beef cattle plays a role in *N. caninum* infection is still controversial. Hornok et al. (2006b) reported that the infection status would get higher when the beef cattle got older. There was a similar finding in which *Bos frontalis* older than 3 years old faced higher risk than those younger (Rajkhowa et al., 2008). By contrast, according to Sanderson et al. (2000), prevalence of infection in 3 years old beef cattle was significantly higher than that in 6 years old. That one year old beef cattle had more probability to be positive than the 3 years old cattle was similarly demonstrated (Garcia-Vazquez et al., 2009). Some other study did not find any effects of the age of beef cattle on the prevalence of the infection (Banales et al., 2006; Damriyasa et al., 2010).

Like previous reports, in the present study, gender did not affect the infection status of the beef cattle. Damriyasa et al. (2010) and Rajkhowa et al. (2008) have also announced the same observation in beef cattle. In sheep, that gender did not influence the prevalence of antibody to *N. caninum* was also reported (Soares et al., 2009). Several similar findings have also been claimed in dogs (Cringoli et al., 2002; Cruz-Vazquez et al., 2008; Haddadzadeh et al., 2007; Vaclavek et al., 2007).

In the present study, herd size was not recorded but the size of herd has been considered a risk factor. Large scale farms increased the prevalence of infection in dairy cattle in Thailand was observed by Kyaw et al. (2004). A scale of more than 25 dairy or beef cattle could produce more risk of infection to the herd (Aguiar et al., 2006). Small dairy farms had 5 times lower danger compared with the large farms (Otranto et al., 2003).

Swamp buffaloes and beef cattle were raised in the same villages in Phu Wiang district, Khon Kaen province. They shared the same rice fields and environment so contact between them was obvious. Presence of the dogs at those villages was available.

The management and feeding were also similar. It is very interesting that the prevalence of the infection in swamp buffaloes (6.4%) was significantly lower than that in beef cattle (43.6%). In Vietnam, proportion of positive dairy cattle (5.5%) was also significantly higher than that of swamp buffalo (1.5%) (Huong et al., 1998). Since there were no studies comparing the susceptibility of swamp buffaloes and beef cattle to *N. caninum*, whether there is any difference between them is unclear. However, there was evidence that the prevalence of *N. caninum* infection was different between breeds of cattle. Quintanilla-Gozalo et al. (1999) found a significantly higher prevalence in dairy cattle (17.9%) than that in beef cattle (36.8%) though they lived at the same location. Several similar results supported the suggestion that dairy cattle are more susceptible to this parasite than beef cattle (Koiwai et al., 2005b; Moore et al., 2002; Otranto et al., 2003). There was also difference within dairy breeds or beef breeds. Jersey cattle had increased odds of being *N. caninum* positive in comparison with Holstein-Friesian cattle (Romero et al., 2002). Similarly, Swedish Red and White breed cattle were more likely to be seropositive than cattle of other breeds (Bartels et al., 2006a). Limousin cattle were found to have less risk of being positive than other dual purpose beef cattle breeds under the same extensive management conditions (Armengol et al., 2007). Also, artificial insemination in dairy cattle using semen of beef cattle, especially Limousin, could reduce the risk of abortion (Almeria et al., 2009; Yaniz et al., 2010). Furthermore, the proof that buffaloes and cattle were differently sensitive to others parasite was available. In a study, buffaloes and cattle were infected with *Trypanosoma vivax* by being either bit by infected tsetse flies or inoculated. While buffaloes showed less local skin reaction, transient of parasitaemia and no signs of anemia, cattle had severe local inflammatory skin reaction, high level of parasitaemia and anemia (Dwinger et al., 1986). In addition, cattle were reported to be less resilient to infection of *Fasciola gigantica* than buffaloes (Molina et al., 2005; Wiedosari et al., 2006). Those clues suggested that the difference in *N. caninum* infection between swamp buffaloes and beef cattle in this study may be due to their different susceptibility to the parasite. The possible explanation for this difference was similar to that between river and swamp buffaloes. There were the substitutions of amino acids in IL-2, IFN-γ, IL-10, IL-1α, IL-1β, IL-6 and TNF-α between swamp buffaloes and cattle (Mingala et al., 2006, 2007) which may lead to the different immunological response to the parasite. On the other hand, the influence of using anti-bovine conjugate for testing buffalo samples on the results has not been known, and could be one of the factors contributing to the finding.

4. Demonstration of *N. caninum* DNA in swamp buffaloes whole blood by Nested-PCR

Polymerase chain reactions are highly sensitive and specific tests to demonstrate DNA of the pathogens. Recently, a nested-PCR has been successfully amplified the DNA fragment in Nc-5 gene of *N. caninum* from the cattle serum (13 positives) which were seronegative (107 samples) in an IFAT test (McInnes et al., 2006b). This finding suggested that the use of PCR technique to examine the DNA of the *N. caninum* in the sera or whole blood, especially the negative samples in serological tests could confer a better view of the *N. caninum* infection status in the animals. The use of whole blood instead of serum in the present study was because of the fact that the DNA of *N. caninum* was usually found in cellular fraction but not in seminal fluid (Caetano-da-Silva et al., 2004; Ferre et al., 2005, 2008). This nested-PCR which can increase 1000 fold of sensitivity compared with the traditional PCR, has been able to detect 1-10fg of genomic DNA, equivalent to 1/10 of a parasite genome (Ellis, 1998). Nc-5 gene of *N. caninum* was identified and used to distinguish the *N. caninum* DNA from its other close parasites such as *T. gondii* and *Sarcocystic* species (Kaufmann et al., 1996). Using of primary primer set of Np21$^+$/Np6$^+$ and secondary primer set of Np9/Np10 could successfully amplify two DNA fragments of 328 bp and 224 bp in Nc-5 gene of *N. caninum* from blood and serum of cattle (McInnes et al., 2006b; Yao et al., 2009).

Amplification of both 328 bp and 224 bp fragments from swamp buffalo whole blood samples was failed. The presence of *N. caninum* in blood of infected bulls was intermittent which could reduce the probability of successful demonstration of *N. caninum* DNA from the blood (Ferre et al., 2005). Practically, the chance to find *N. caninum* DNA in biological fluids was not high. Out of 813 cattle serum samples, only

0.9% of them was positive in a similar nested-PCR test while the antibodies to *N. caninum* were found in 15.5% samples by an ELISA (Yao et al., 2009). The low probability of a successful amplification of *N. caninum* DNA in serum may be attributable to the hypothesis that the parasites circulate intra-cellularly in leucocytes but not in the plasma since the circulating antibodies could easily kill them in serum (Okeoma et al., 2004a). The failure of amplifying the *N. caninum* DNA may be due to the low or absence of DNA of parasite in the blood since collection of blood may occur when the infection was in chronic stage. Moreover, the parasite were mostly found in brains, spinal cords, hearts, livers or muscles (Dubey and Schares, 2006; Kul et al., 2009; Pabon et al., 2007; Sanchez et al., 2009).

In the infected bulls, intermittent detection of *N. caninum* DNA in blood and semen was reported (Ferre et al., 2005). Those authors weekly collected semen and blood samples from 8 infected bulls in 22 successive weeks, only 9.8% and 7.9% semen and blood samples were positive in PCR test, respectively. Throughout their study period, only 62.5% (5/8) infected bulls had positive semen or blood. Similarly, the presence of *N. caninum* DNA was found only in 3.7% and 9.1% blood and semen samples from experimentally infected bulls, respectively (Serrano-Martinez et al., 2007a). The efficiency of the test that they used was stated to be able to detect 1 tachyzoites in 250μl of artificially-contaminated semen (Caetano-da-Silva et al., 2004; Ortega-Mora et al., 2003).

Furthermore, even in one ejaculation of infected bulls, DNA of *N. caninum* was not constantly successfully uncovered. From 9 positive samples in 9 ejaculations of 3 bulls, an addition of 3 samples of each ejaculation was further studied, only one sample out of these 27 samples was found to contain *N. caninum* DNA (Caetano-da-Silva et al., 2004). The intermittent presence of *N. caninum* in blood and semen may be due to the fact that the samples were collected in different stage of infection, the lack of presence of DNA may be due to the chronic stage (Caetano-da-Silva et al., 2004). At this stage parasite are located as dormant form as bradyzoite in tissue cysts in the central system and probably in muscles (Dubey, 2003; Dubey and Lindsay, 1996). In the present study, samples were collected when there was no outbreak of abortion and the serological test results also suggested no outbreak of infection. It could be presumed that the infection, if there was, was in chronic stage and parasites were in the inactive form as bradyzoites, or did not travel in blood at a detectable level. In addition, if there were parasites in the blood, they could be killed by the antibodies. This might be one of explanations to the finding that two samples which were positive in the ELISA test were negative in the PCR test.

Unsuccessful amplification of *N. caninum* in the present study may be due to the low amount or absence of parasite DNA in the buffalo whole blood. In this study, the whole procedure of DNA extraction was carried out by using standardized kits (QNeasy-QIAGEN) or the use of TRIZOL reagents which was routinely carried out in this laboratory. In addition, PCR technique was running regularly. However, if the positive control had been put into each amplification, the interpretation of the results would have been more confident.

In the present study, although prevalence of *N. caninum* infection in swamp buffaloes in Northeast Thailand was lower than which had been reported in dairy and beef cattle in the country, it conveyed a new information that swamp buffaloes in Thailand were also infected with *N. caninum*. The present study detected an interesting finding that beef cattle were more likely to be susceptible than the swamp buffaloes to *N. caninum*. The effects of neosporosis on the reproductive performance of livestock are obvious though this study was not able to uncover the impact of this disease on such criteria of swamp buffaloes. Prospective study focusing on the full gestation would be a better choice for exploring the influence of *N. caninum* infection on the reproduction of swamp buffaloes. PCR test using of sample from aborted fetuses or aborting infected dams, repeated sample collection and larger sample size from the infected swamp buffaloes would increase the chance of successfully amplifying *N. caninum* DNA.