รายงานวิจัยฉบับสมบูรณ์

การศึกษาความชุกและปัจจัยเสี่ยงต่อการแพร่กระจายของการเกิดโรค ของโรคนีโอสปอโรซิสและโรคทอกโซพลาสโมซิสที่ทำให้เกิดการแท้ง ใน โคนมในเขตภาคตะวันตก

โดย

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A Study to Estimate the Prevalence and Risk factors of Neosporosis and Toxoplasmosis in Dairy Cattle in Western Thailand

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Abstract

The objective of this study was to investigate the seroprevalence and risk factors of *Neosporosis* and *Toxoplasmosis* in dogs, cats, and dairy cows of Western part of Thailand. The first year period. a total of 300 dairy sera from 60 dairy herds from Nakhon Pathom, Ratcahburi, Kanchanaburi provinces were collected. Neosposis was estimated using a commercial antibody-detection ELISA, and examined for antibodies against *T. gondii* infections using a latex agglutination test. Of these, 9.7% (29/300) was found to be positive to *N. caninum* and herd prevalence was 35% (21/60). Seroprevalence of *T. gondii* was 7% and antibody titers ranged from 1:64 to 1:512 (cutoff, 1:64). Herd prevalence was 26.7% (16/60). No significant difference was observed among the provinces. The present of chicken had 2.7 times greater odds for being seropositive for *N. caninum* (P=0.014). The herds had mouse ran around in farms was 3.5-times more likely to be positive. The risk factors associated with *T. gondii* infections were a presence of cat in farms (OR = 14.0, P=0.01) and there was relationship between toxoplasmosis and reproductive problem in dairy farm (OR =3.6, P=0.05). For the second year, dogs and cats form positive farms were collected as case and pets from neighbourhood frams were randomly selected as control, totally 40 herds. Of the 114 dogs and 36 cats sera sample tested, 8 sera samples from dogs (7%) were seropositive to *N. caninum.* No cats were found positive. Of the 55 farm dogs, 6 (10.9%) were seropositive to *N. caninum,* which was significantly higher than the proportion of seropositive in the negative herd population (2 of 95, 2.1%). The higher proportion of seropositive farm dogs as compared with neighbourhood dogs was statistically significant (P=0.021). Seven sera samples from dogs (6.1%) and 3 sera samples from cats 8.3% were found the titer of *T. gondii* range from 1:64 to 1:256 in dogs and from 1:256 to 1:2048 in cats. The dogs older than 1 year was 9 times more likely to be positive for *Neospora caninum* than younger dogs in the study (P=0.04). The feces contamination was 4.6 times trend to be positive in the study. In the positive household, the pregnancy abnormality of the owner was found 7.7 times more likely than negative house. The results of *N. caninum* and *T. gondii* infections indicated that dairy cows in Western provinces were exposed to both diseases. Therefore, it is possible that biosecurity within a farm is important to control horizontal transmission of these agents. The further research on Neosoposis in wildlife canids is needed to find the status of this disease in Thailand wildlife and the vertical transmission which the major source of transmission in dairy herd should be monitored.

Keywords : Toxoplasmosis, Neosporosis, Dairy cattle, Dogs, Cats, Risk factors

การศึกษาความชุกและปัจจัยเสี่ยงต่อการแพร่กระจายตัวของโรคนีโอสปอโรซิสและโรคทอกโซพลาสโมซิสที่ทำให้เกิดอาการแท้งในโคนมในเขตภาคตะวันตก

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บทคัดย่อ

จุดประสงค์ของการศึกษาเพื่อศึกษาความชุกและปัจจัยเสี่ยงต่อการเกิดโรค *Neosporosis* และ *Toxoplasmosis* ในสุนัข แมว และโคนม ในเขตภาคตะวันตก ในปีแรกทำการเก็บตัวอย่างโคนมจำนวน 300 ตัวอย่างจาก 60 ฟาร์มในจังหวัดนครปฐม ราชบุรี และกาญจนบุรี โดยนำซีรั่มของโคนมมาทดสอบด้วยวิธี c-ELISA สำหรับโรคนีโอสปอโรซิส และวิธี latex agglutination test สำหรับการทดสอบการติดเชื้อ *T. gondii* จากผลการทดสอบพบความชุกของโรคนีโอสปอโรซิสในระดับรายตัว 9.7% (29/300) และในระดับฝูง 35% ความชุกต่อเชื้อ *T. gondii* คิดเป็นร้อยละ 7.0 โดยระดับไตเตอร์อยู่ในช่วง 1:64 ถึง 1:512 (ค่า *cut off* = 1 : 64 หรือมากกว่า) และค่าความชุกของโรคในระดับฝูงพบร้อยละ 26.7 (16/60) ไม่พบความแตกต่างของการเกิดโรคในแต่ละจังหวัด การเลี้ยงสัตว์อื่นภายในฟาร์ม (ไก่) เป็นปัจจัยเสี่ยงโดยมีโอกาสการเป็นติดเชื้อ *N. caninum* เป็น 2.7 เท่า (P=0.014). การพบหนูในฟาร์มจะมีแนวโน้มในการติดโรคมากกว่า 3.5 เท่า ปัจจัยเสี่ยงต่อการติดเชื้อ *T. gondii* ในฟาร์มโคนมได้แก่ การพบหรือมีแมวเลี้ยงในฟาร์ม (OR=14.0, P=0.01) และพบความสัมพันธ์ของโรคและปัญหาระบบสืบพันธุ์ในฟาร์ม (OR = 3.6, P=0.05) ในปีที่สองทำการศึกษาในสุนัขและแมว ในฟาร์มที่ให้ผลบวกต่อโรคและฟาร์มข้างเคียงจำนวนรวม 40 ฟาร์ม จำนวนสุนัข 114 ตัวและแมว 36 ตัว ความชุกในสุนัขในฟาร์มเท่ากับ 7% (8/114) ไม่พบแมวให้ผลบวกต่อโรคนีโอสปอโรซิส สุนัขจำนวน 6 ตัวจาก 55 ตัวในฟาร์มที่ให้ผลบวกต่อโรคในโคนมคิดเป็น 10.9% ซึ่งสูงกว่าและแตกต่างอย่างมีนัยสำคัญทางสถิติ เมื่อเทียบกับฟาร์มข้างเคียงที่ไม่เป็นโรค (P=0.021) ความชุกของโรคทอกโซพลาสโมซิสในสุนัขและแมวพบร้อยละ 6.1และ 8.3 ตามลำดับ โดยค่าไตเตอร์อยู่ระหว่าง 1:64ถึง 1:256 ในสุนัขและ 1:256 ถึง 1:2048 ในแมว ในสุนัขที่มีอายุมากกว่า 1 ปี มีโอกาสติดโรคนีโอสปอโรซิสสูงกว่าสุนัขน้อยถึง 9 เท่า (P=0.04) การปนเปื้อนของอุจจาระมีแนวโน้มในการติดโรคทอกโซพลาสโมซิสสูงถึง 4.6 เท่าและคนในฟาร์มที่ให้ผลบวกต่อโรคพบความผิดปกติของการตั้งครรภ์สูงถึง 7.7 เท่าเมื่อเทียบกับฟาร์มที่ไม่เป็นโรค จากผลการศึกษาสรุปว่า โรคนีโอสปอโรซิสและทอกโซพลาสโมซิส มีการแพร่กระจายในฟาร์มโคนม และสัตว์เลี้ยงในฟาร์มโคนมในเขตภาคตะวันตก ดังนั้นการป้องกันทางชีวภาพจึงเป็นสิ่งจำเป็นในการป้องกันการติดโรค และควรมีการศึกษาเพิ่มเติมในสภาวะโรคของสัตว์ป่าและการติดเชื้อแบบแม่สู่ลูกในโคนมต่อไป

คำสำคัญ : โรคทอกโซพลาสโมซิส โรคนีโอสปอโรซิส โคนม สุนัข แมว ปัจจัยเสี่ยง

Introduction

Neospora caninum is an apicomplexan protozoan that was first recognized in dogs in Norway in 1984 (Bjerkas, 1984). In 1988, a new protozoan species, *N. caninum* was proposed under a new genus, *Neospora* (Dubey, 1988). It is one of the most efficiently transplacentally transmitted organisms in cattle (Dubey and Lindsay, 1992). The most important route of infection in cattle is transplacental transmission (Bjorkman et al. 1996). Horizontal transmission also occurs by ingesting feed and water contaminated with *N. caninum* oocysts shed in the feces of dogs as seen in Fig 1(McAllister et al., 1998, Lindsay et al., 1999). Cows with *N. caninum* antibodies (seropositive) are more likely to abort than seronegative cows and this applies to both dairy and beef cattle (Lopez-Gatius, et al. 2004). Moreover, cows that abort due to *N. caninum* are more likely to abort due to *N. caninum* again in the future (Moen et al. 1998). Most neosporosis-induced abortions occur at 5-6 month gestation. A case control study of 83 herds in Ontario, Canada, showed that 305-day milk production for seropositive cows was 250 to 300 kg less than for seronegative cows in herds with a history of *N. caninum* abortion problem (Hobson et al. 2002). There is no cow-to-cow transmission of *N. caninum.* Examination of the serum from an aborting cow is only indicative of exposure to *N. caninum*. There is a rise in antibody titers 4 to 5 months before parturition. It is likely that there is parasitemia during pregnancy leading to fetal infection. *Neospora caninum* infected calves may have neurologic signs, be underweight, unable to rise, or be born without clinical signs of disease. Infected calves are seropositive and may remain infected and seropositive for life (Pare et al., 1996) However, *N. caninum* has never been identified in histologic sections of adult cows and there is a single report of isolation of viable *N. caninum* from the brain of an adult cow (Sawada et al., 2000).

Neosporosis has emerged as a serious disease of cattle and dogs worldwide (Dubey, 1999). It is a major cause of abortion in dairy cattle in the U.S. (Anderson et al., 1995). A high prevalence of neosporosis in dairy cattle which can be up to 87% in Netherland's dairy herds (Wouda et al, 1999). Based on serological surveys, up to 100% of cattle in some herds have been exposed to *N. caninum* (Dubey and Lindsay, 1996). It also has been reported in water buffaloes in Egypt (Dubey et al., 1998b), in cattle and water buffaloes in Vietnam (Huong et al., 1998) and cattle in Thailand (Suteeraparp et al., 1999, Kashiwazaki, et al. 2001,Chanlun et al. 2002, Kyaw et al., 2004). At present there is no evidence that *N. caninum* successfully infects humans. Although cats can be easily infected with *N. caninum* experimentally (Dubey et al., 1989), it was a few reports of natural exposure of cats to *N. caninum* (Dubey et al., 2002)

Seroprevalences of 12–70% in dairy cattle have previously been reported in northeast Thailand by IFAT at different cut-off levels (Suteeraparp et al., 1999; Kashiwazaki et al., 2001). Chanlun et al (2002) found that 81.8% (nine of eleven herds from Northeast areas) were seropositive from bulk tank samples. The seroprevalence from individual cows averaged 15% (24/164) and ranged from 0 to 46% in the eleven herds. One study showed individual and herd seroprevalence of *N. caninum* were 5.5% and 34% which was done in two sub-districts in Amphur Meung, Nakhon Pathom (Kyaw, 2004). The small sample sizes in the 2 sub-districts certainly cannot be considered representative of the cow population in these provinces. Suteeraparp et al. (1999) reported the results

from a survey of dairy cattle in Nakhon Pathom Province, and found 4 positive from 58 (6.9%), while the seroprevalence in central Thailand was 6%.

Neospora seropositivity in cattle is associated with many risk factors, such as the presence of dogs on the farm, the presence of poultry, and the feeding of moldy fodder. However, little information is available on the epidemiological factors that might explain high seroprevalence.

Toxoplasma gondii is a ubiquitous protozoal parasite of warm-blooded animals (Dubey, 1998a). It has an extremely broad host range including birds, wild animals, pets, livestock and humans (Fig. 2). *Toxoplasma. gondii* is prevalent in most areas of the world Environmental contamination with *T. gondii* oocysts extends even into the oceans. High *T. gondii* seroprevalence rates were recently demonstrated in a variety of marine mammals (Dubey et al., 2003). *Toxoplasma. gondii* is of veterinary and medical importance, because it may cause abortion or congenital disease in its intermediate hosts. In human, infections in most immunologically normal humans are asymptomatic or result in an influenza-like illness, which often goes undiagnosed as Toxoplasmosis (Tenter et al, 2000). Women who become infected for the first time during pregnancy, and immunosuppressed patients are at serious risk from toxoplasmosis. *Toxoplasma* infection can be life threatening in congenitally infected and immunosuppressed patients (Chintana et al., 1998) *Toxoplasma gondii* is recognised worldwide as a major cause of morbidity and mortality in AIDS patients, primarily as a result of encephalitis (Luft el al. 1992). Humans usually become infected with *T. gondii* by ingesting tissue cysts from undercooked meat or by ingesting food or water contaminated with oocystes from infected cat feces (Dubey, et al., 2004). In animals, *T. gondii* infection not only results in significant reproductive and hence economic losses, but also has implications for public health since consumption of infected meat or milk can facilitate zoonotic transmission (Hill, et al., 2005)

In Thailand, studies on the seroprevalence of *T. gondii* infection have been performed in humans with 1.2-4.6% (Maruyama, 2000), cats with 7.3% (Sukthana et al, 2003) and 11% (Jittapalapong 2007), dogs with 3.5% (Nishikawa et al,. 1989), and 9.4% (Jittapalapong 2007), goats with 27.9% (Jittapalapong, 2005), The overall seroprevalence of *T. gondii* was found in 21 of 136 (15.4%) captive felids of 12 species (Thiangtum et al., 2006), and elephants with 25.6% (Tuntasuvan et al 2001). Suteeraparp et al. (1999) reported the results from a survey of dairy cattle in Nakhon Pathom Province, and found 4 *Toxoplasma* seropositive samples (1:64 <) from 119 samples (3.4%) in central Thailand. The prevalence for *T. gondii* antibodies in southern Vietnam was 10.5% (21/200) (Huong et al. 1998). However, *T. gondii* infection in meat-production animals, particularly dairy cows in the western Thailand is questionable to date.

The little information available on the epidemiology of Neosporosis and Toxoplasmosis in Thailand is in Western Thailand. Therefore, further studies are needed to investigate the epidemiological status of the disease so that preventive and control measures can be implemented and monitored. Moreover, the little information available on the epidemiology of Toxoplasmosis in dogs and cats in dairy farms in Thailand. Therefore, the purpose of this study was to determine seroprevalence and the risk factors transmitted of *N. caninum* and *T. gondii* in dairy cows and dogs and cats in dairy farms in Western part Thailand**.** This data will be beneficial for prevention and control for

veterinarians work in the areas and will help save Thai farmers to reduce the cost of livestock production.

Materials and Methods

Animals and blood collection

The study was carried out between June 2006 and May 2008 (2 year periods) as seen in Fig 1. On the first year, the area selected for sample collection was in western part of Thailand composed of Nakhon Pathom, Rachaburi, and Kanchanaburi province (Fig 2), where 3 of top 10 highest dairy population areas in Thailand. Most of the dairy farms in this area were intensively reared. Blood was randomly collected from 20 dairy farms in each province, totally 60 herds in the study. Five dairy cows from each herd were randomly selected with total of 300 samples. Data were obtained using a questionnaire, regarding the state of herd management and was administered on site, including animal species present. All dairy cows were bred for milk production. The majority of the cattle were cross bred Holstein Friesian. Most of dairy herd in the study was a small holder range 5-20 milking cows. The total number of lactating cows in these herds ranged from 5 to 80. Most of them were tie stall barn with component feeding. The blood was collected by caudal venepuncture, and centrifuged at 1000 x g for 10 min and the serum separated and stored at -20˚C until serologic analysis.

On the second year, blood samples of dogs and cats in positive farms in Nakhon Pathom, Rachaburi, and Kanchanaburi province were collected as case. Dogs and cats from neighbourhood farms were randomly selected as control. Totally, 114 dogs and 36 cats in the study. These samples were performed between June 2007 and May 2008 (year 2). The questionnaire of pets was obtained in each herd visit, including information on type, age, sex, feeding habits with raised on farm. The blood was centrifuged at 1000 x g for 10 min and the serum separated and stored at -20˚C until prior testing.

Serological assay for Toxoplasma gondii

Antibodies to *T. gondii* were examined by commercial latex agglutination test kits (TOXOCHECK-MT; Eiken Chemical, Tanabe, Tokyo, Japan). This test was evaluated as a screening serologic test for toxoplasmosis in animals (Tsubota et al., 1977a and b). The procedure described in a previous report (Maruyama et al., 2003) was followed accurately. Briefly, twenty-five microliters of latex agglutination buffer was added to each well of a U-shaped 96 well cluster plate. Then 25 µl of 1:8 diluted sera was mixed with the buffer in the first well. Serial two-fold dilutions were performed in all wells and the final 25 µl was discarded. Then 25 µl of *T. gondii*-antigen-coated latex beads were added to each well. The plate was shaken gently and then incubated at room temperature overnight. The cutoff titer for this test was 1:64 according to the manufacturer's instructions in the kit and were further diluted to determine the end point.

Serological assay for Neospora caninum

Sera from 300 cows were collected for antibodies against neosporosis. Competitive enzyme-linked immunosorbent assay (c-ELISA, VMRD, USA) was performed as the

diagnosis of *N. caninum* infection. Fifty microlitter of serum samples were transfer into antigen-Coated plate for each sample including negative and positive control. The plate was incubated at room temperature for 1 hr then wash with washing buffer solution for 3 times. Antibody-Peroxidate conjugate (50 µl) was added to each well and incubated for 20 minutes at room temperature and washed with washing buffer solution 3 times. Fifty microlitter of substrate were added into the well and incubated for 20 minutes then added stop solution for stop reaction of the ELISA. Finally , the plate was measure the optical density at 630 nm immediately

Statistical analysis

Statistical analysis of descriptive data were performed, using logistic regression. We determined unconditional associations between disease status and each variable (using Wald's test, $P < 0.20$, two-sided). In dairy data composed of the present of cats, dog, mouse, other animals (chicken) in farm, herd size (small (10-40)-large (>40), barn type (tie stall, free stall and both), parity (0-9), reproductive problem, abortion in farms, provinces. In pet data compose of sex (male, female), age (≤ 1 year, >1 year), pet born in farm or not, illness status, pet died during one year period, feces contamination in dairy feed, owner drinking raw milk, pregnancy abnormality in owner). We also calculated simple correlation coefficients among all variables to determine if independent variables were highly correlated. When two unconditionally significant variables were highly correlated, the variable with the strongest association and best biological plausibility was selected to be included in the final modeling procedures. The final multivariable random-effects logistic regression model was developed using a backward process of eliminating least significantly associated variables until all remaining variables were significant at $P < 0.05$ (two-sided), based on the likelihood ratio chi-square test. Finally, to improve the model fit, transformations of significant main effects in the model were order. Goodness-of-fit of the final model was examined to assess how well the model fit the observed data. For the positive status of antibodies to *N. caninum* in farm dogs was analysed in relation to the positive status of neosporosis in the cattle on the farms using the chi-square test and the Mantel extension test for trend ($P < 0.05$) was considered significant. All analyses were conducted using the statistical software package STATA (version 8.2, Stata Corp., College Station, TX)**.**

Results

Neosporosis and Toxoplasmosis in Dairy cattle

As seen in Table 1, twenty nine animals (9.7 %) were positive to *N. caninum*. The seropositive rates of dairy in region Kanchanaburi, Nakhon Pathom and Ratchaburi were 6%, 10%, and 13% respectively. Herd prevalence was 35% (21/60). Ratchaburi has the most positive herds with 45.0% (9/20), 40.0% (8/20) in Nakhon Pathom, and 20.0% (4/20) in Kanchanaburi, respectively. Of the 300 dairy sera sample tested, 21 (7%) were found the titre of *T. gondii* range from 1:64 to 1:512, (range 1:64, 1:128, 1:256 and 1:512 amount 14, 4, 1 and 2 samples, respectively). The seropositive rates of dairy in region Kanchanaburi, Nakhon Pathom, and Ratchaburi were 3%, 9%, and 9% respectively, and the average was 7%. The herd prevalence in Nakhon Pathom was 40% (8/20), 15% (3/20) in Kanchanaburi, and 25% (5/20) in Ratchaburi with totally 26.7% (16/60). No significant difference was observed among the provinces. Four dairy

cows were positive both diseases. All *N. caninum* positive results were seen in all lactations (1-9), including pregnant heifer. *Toxoplasma gondii* titer positive were seen in younger dairy cows than older parity (Table 2).

The simple correlation coefficients among all variables in analyses of neosporosis were not correlated. The present of dogs or cat, reproductive problem in farms, parity in cows and province were not significant association in unconditional variables as seen in Table 3. Herd size, the present of mouse and other animals (chicken), barn type were significant in unconditional analysis. There were two variables that fit in the final model. The present of other animals (chicken) was significant difference in *N. caninum* seropositive cattle on this study, and present of mouse in farm trend to be a horizontal transmission factor in farm (Table 4).

The simple correlation coefficients among all variables in analyses of toxoplasmosis were not correlated. The present of dogs, mouse, other animals, herd size, barn type, abortion in farm and parity were non-significant association in unconditional variables as seen in Table 3. There were significant difference between the present of cat and the present of *T. gondii* seropositive cattle on this study. Reproductive problem was significant. Table 5 showed the risk factors in the final model.

Neosporosis and Toxoplasmosis in Dogs and Cats

All *Neospora cani/num* positive results were found in dogs as seen in Table 6 with over all seroprevalance 7% (8/114). The seropositive rates of dogs for neosprosis in region Kanchanaburi, Nakhon Pathom and Ratchaburi were 10.0%, 4.2%, and 7.1% respectively, No cats were found positive. The 21 positive dairy herds were followed up to collect their pets' samples. Fourteen dairy herds from 21 positive herds were collected, 4 positive herds had no dogs in farms, and dogs from 3 positive herds died before collecting samples. Four herds from fourteen herds had positive dogs in their farms. Of the 55 farm dogs, 6 (10.9%) were seropositive to *N. caninum,* which was significantly higher than the proportion of seropositive in the negative herd population (2 of 95, 2.1%). The higher proportion of seropositive farm dogs as compared with neighbourhood dogs was statistically significant (P = 0.021).

For Toxoplasmosis, of the 114 dogs and 36 cats sera sample tested, 7 sera samples from dogs (6.1%) and 3 sera samples from cats 8.3% were found the titer of *T. gondii* range from 1:64 to 1:256 in dogs and from 1:256 to 1:2048 in cats as shown in Table 7. The seropositive rates of dogs and cats for toxoplasmosis in region Kanchanaburi, Nakhon Pathom and Ratchaburi were 14.8%, 5.1%, and 4.7% respectively, and the average was 6.7%. The Toxoplasmosis herd prevalence in Kanchanaburi was 28.6% (2/7), 33.3% (4/12) in Nakhon Pathom, and 14.3% (3/21) in Ratchaburi with totally 22.5% (9/40) as similar to Neosporosis 25% (2/7), 8.3% (1/12) and 19% (4/21) in Kanchanaburi, Nakhon Pathom and Ratchaburi, respectively, with totally 17.5%.

The simple correlation coefficients among all variables were not correlated. There were one variable, age, significant in neosporosis data as seen in Table 8. The dogs older than 1 year was 9 times more likely to be positive for *N. caninum* than younger dogs in the study (P=0.04). For Toxoplasmosis, there were two variables in the final model but the model was not fit. Therefore, the interpretation was described as unconditional model. The ingesting food or water contaminated with oocystes from infected cat feces

is a major route of transmission in this study. The feces contamination was 4.6 times more likely to be positive in the study. In the positive household, the pregnancy abnormality of the owner was found 7.7 times more likely than negative household. Women who become infected for the first time during pregnancy, and immunosuppressed patients are at serious risk from toxoplasmosis.

Discussion

Neosporosis in dairy cows

Our observations found that 35% (21/60) of the herds studied had at least one animal seropositive to *N. caninum* suggested that neosporosis was widespread among dairy herds in western Thailand. Ratchaburi is the most positive cows with 13% (13/100) and also herds with 45 % (9/20). The overall prevalence of *N. caninum* was 9.7 % (29/300). The result was similar to the current study by Jittalapong (2008) who reported the seroprevalence in the North-east with 11.7%. The results of *N. caninum* infections indicated that dairy cows in Western provinces were exposed to *N. caninum* and the prevalence was higher than the previous serological survey (6%) in the central part. This increase may be partially due to a difference in the serological tests used and among study subjects in this study and earlier surveys. Moreover, the study from Kyaw et al;, (2004) who study in two sub-district in Nakhonpathom found individual and herd prevalence were 5.5% (30/549) and 34% (20/59) with the same tests. When compared to our study at the same province found higher than previous study with 10% (10/100) individual and 40% (8/20) herd prevalences. This was indicated the extent of infection or exposure among the dairy cattle population seem to be increased. All positive results were seen in all lactations, including pregnant heifer. Parity is not significant in the study.

It has been demonstrated that dogs can act as definitive hosts of *N. caninum*. Three of 4 dogs that were fed mouse tissues containing *N. caninum* tissue cysts shed fecal oocysts (McAllister et al. 1998). Dogs are suspected to play an important role in horizontal transmission, especially point-source epidemic exposure of cattle to *N. caninum* (McAllister et al.,2000; Bartels et al., 1999). The presence and the number of dogs are correlated to high seroprevalence of *N. caninum* in dairy cattle (Paré et al., 1998;Wouda et al., 1999). In contrast, the present of dogs in farm is not significant in this study with similar to previous study (Kyaw et al., 2004). Probably, the dogs that present in these study herds might be still free of diseases. It is very interesting to examine the disease status of dogs in the positive herds as described next. Chanlun et al. (2006) studied in antibody status in 418 dairy herds in northeast for three consecutive sampling. Eighty six percent of the 158 herds were considered negative at sampling 1 and sampling 2 remained negative at sampling 3. Herd can keep a negative infection status in dairy cows despite the frequent presence of dogs.

Seropositive cows trend to have the higher risk of abortion than seronegative animals (OR= 2.4, P = 0.08), suggesting that *N. caninum* might be one of the causes of abortion in dairy cows in Thailand as same as the one of the major cause of bovine abortion in several countries (Anderson et al., 1991; Davidson et al., 1999; Lopez-Gatius et al., 2004; Moen et al., 1998). It was similar to report from British study. Davidson et al (1999) found a strong association between seropositivity and abortion with seropositive cows being 3.5-times more likely to abort than seronegative cows (OR=3.49, $P < 0.05$).

Wouda et al., (1998) found that seropositive F 1 cows had a three-fold increased abortion risk compared with seronegative F 1 cows, suggested that *N. caninum*-infected calves should not be used as replacement stock, to decrease the future risk of abortion in dairy herds.

Herd size is a significant variable in the unconditional analysis. The big herd (>40 dairy cows) was 2 times more likely to had seropositive dairy cows. Not surprise, it was not in final model. Dairy cow is intermediate host and can not produce oocysts in her body. The spread between cows to cows is not possible. Therefore, vertical transmission might be the main source of neosporosis infection and may contribute significantly to the persistence of the infection in the herd (Pare et al., 1996). Normally, cows in large farms had a longevity than small herds due to the good herd management. Once cows get infected with *N. caninum* either by horizontal or vertical infection they will probably remain persistently infected (Anderson et al., 1997). Therefore, herd may be a confounder in the study.

Cows that fed in free stall barn was 2 times more likely to be positive than that tie stall barn. Some herds are allowed to graze even though fresh grass is usually cut, carried and given to the cattle during the rainy season, making more chance to contact with the oocysts than the cows that normally keep in barn.

It seems to be that there are other ways of horizontal transmission as mouse and other animal in farm (chicken) which roam and run around in farms. These two variables were still significant in the final model. Bartels et al. (1999) found the presence of poultry on the farms as one risk factor in abortion storm in dairy herds in Netherlands. These animals could serve as vectors of oocysts, particularly when they are grubbing about freely. In addition, poultry might play a role as an intermediate host by which a dog may become infected (eating dead fowl). In the study, mouse was trend to be another main source of horizontal transmission, the farms that have present of mouse roam and run around in farms, especially in feeding area was 3.5-times more likely to be a positive herd. Lindsay et al (1999) showed the evidence of dogs can infected *N. caninum* by eating mouse brains containing tissue cysts of the *N. caninum*, and excreted ooysts.

Neosporosis in dogs and cats

The present of antibodies in the dogs in farm was also investigated in the study to determine the relation of prevalence of antibodies to *N. caninum* in farm dogs and the cattle on the farm. We found 7% (8/114) seroprevalence in dogs which higher than previous study 1.2% (1/82) seroprevalence of farm dogs in Nakhon Pathom province (Kyaw et al., 2004). This was indicated that this area was more endemic and high seroprevalence in dogs and cattle.

No cats were found positive, indicating that no natural exposure in our study. However, it was a few samples of cats in this study, it might be some exist elsewhere. Dubey et al., (2002) report the first documentation of natural exposure of cats to *N. caninum* in Brazil. The samples were collected during 1993 to 2000. Antibodies to *N. caninum* were found in 60 (11.9%) of 502 cats with titers of 1:40 in 36 cats, 1:80 in 18 cats, 1:160 in 5 cats, and 1:800 using direct agglutination tests. However, *N. caninum* has not yet been demonstrated in tissues of naturally infected cats.

Of the 55 farm dogs, 6 (10.9%) were seropositive to *N. caninum,* which was significantly higher than the proportion of seropositive in the negative herd population (2 of 95, 2.1%). The higher proportion of seropositive farm dogs as compared with neighbourhood dogs was statistically significant (P = 0.021). Seropositivity to *N. caninum* of farm dogs was correlated with a high seroprevalence of neosporosis in the cattle. Dogs are common as a pet and have easy access to cattle, pasture and feed storage rooms. Not only the farm dogs, but also the stray dogs or the dogs from nearby-farms may be a considerable factors in the spread of infection in areas where many farms are established very close to each otherThe highest seroprevalences in positive farm dogs (10.9%) compared with neighbourhood farm dogs (2.1%) may be accounted for by the fact that dogs may acquire infection by ingesting cattle tissues, fetal fluids or placental material of infected cattle (Dijkstra et al., 2001, 2002) or considering that farm dogs are more likely to show their carnivorous behaviour by eating small mammals and birds which possibly contain *N. caninum* tissue cysts (Bartels et al., 1999), and may subsequently cause a post-natal infection of cattle in the herd by shedding oocysts.. Gondim (2002) found that dogs fed with tissue from *N. caninum*-infected calves shed up to 503,300 oocysts. This finding demonstrates that at a single time-point a dog can shed large number of oocysts into the farm environment, which may then infect many animals in the herd, including chicken and mouse.

Seroprevalence was higher (2 times) in female dogs than in male dogs, but not significant in the study with similar to Brazilian study. Gennari (2006) found that female dogs had a higher prevalence of antibodies for *N. caninum,* 20.4% (20/98), than that observed for males, which was 15.1% (16/106). However, the chi-square test did not show an association between sex and the presence of anti-*N. caninum* antibodies in the population (P = 0.320). In controversy, Wouda (1999) found that seroprevalence was significantly higher (P = 0.01) in female dogs than in male dogs. The observation that relatively more female dogs than male dogs were seropositive might suggest that recrudescence of infection and a subsequent rise of antibodies is more likely in females.

The dogs older than 1 year was 9 times more likely to be positive for *Neospora caninum* than younger dogs in this study with agree to European studies (Wouda et al., 1999; Paradies et al., 2007). Wouda (1999) and Paradies (2007) reported that the prevalence of *N. caninum* antibodies in dogs increases with dog's age, which indicates that the infection is mainly maintained by horizontal (either oocysts or infected tissues) rather than vertical infection. Gondim (2005) found that a dog's age and type of tissue can influence oocyst production.

In conclusions, the results of *N. caninum* infections indicated that dairy cows and farm dogs in Western provinces were exposed to *N. caninum* with found in high herd prevalences. The present of mouse and chicken were the risk factors associated with *N. caninum* infections in dairy herds in the study. Seropositivity to *N. caninum* of farm dogs was correlated with a high seroprevalence of neosporosis in the cattle and that dogs at higher risk of exposure are free-ranging ones living in farms. Therefore, it is possible that biosecurity within a farm is important to control horizontal transmission of this agent. The further research on Neosoposis in wildlife carnivore is needed to find the status of this disease in Thailand wildlife and the vertical transmission which the major source of transmission in dairy herd should be monitored.

Toxoplasmosis in dairy cows

In general, seropositivity to *T. gondii* in cattle is not high. Infection in cattle does not usually cause clinical symptoms, since cows have a high natural resistance to *T. gondii.* Dubey (1985) recorded a seropositivity of 3.2% to *T. gondii* antibodies among cattle in Montana using a modified agglutination test and sera previously tested with 0.2 M-mercaptoethanol. The prevalence for *T. gondii* antibodies in cattle in Brazil was 1.03% (2/194) (Gondim et al., 1999) and southern Vietnam was 10.5% (21/200) using the LAT (Huong et al. 1998). In our study the frequency of antibodies to *T. gondii* observe in dairy cows was higher (7%) than previous report (3.4%) by Suteeraparp (1999). This increase may be partially due to a difference in the serological tests used and among study subjects in the study. Furthermore, in companion study (Jittapalapong et al., 2008) found the 22.3% seroprevalence in northeastern Thailand that was conducted in 445 dairy cows from 55 farms. The high prevalence of *T. gondii* in dairy cows has potentially important implications for public health. Bovine abortion due to *T. gondii* could result in a reduction of milk production and culling of animals, and thus substantial economic loss.

On the positive herds had cats as pet and no boundary between dairy farm and their houses. Cats are capable of roaming all areas including food storage and even in barns. Our study found that the risk factors associated with *T. gondii* infections were a present of cat in farm (OR = 14, P = 0.009)**.** Domestic cats are the main source of *T. gondii* contamination of cities, farms, and farm animals. Cats play an important role in the spread of toxoplasmosis because they are the only animals that excrete resistant oocysts into the environment (Silva et al., 2001). House cat are common in Thailand. The results of an antibody survey in domesticated cats in Thailand showed a 10.8% prevalence, as reviewed by Jittapalapong et al. (2007). Cats shed oocysts after ingesting any of the three infectious stages of *T. gondii*, i.e., tachyzoites, bradyzoites, and sporozoites. Oocysts of *T. gondii* are formed only in felids, probably in all members of the Felidae. Oocysts are the environmentally resistant stage in the life cycle of coccidia (Hill et al., 2005).

In intermediate hosts, *T. gondii* does not produce oocysts but instead forms cysts in muscular or neural tissues, causing latent toxoplasmosis. The life cycle is completed when a felid ingests infected tissue. Intermediate hosts can also acquire infection through this route and by transplacental transmission (Tenter et al., 2000). *Toxoplasma spp.* is typically not highly virulent, being primarily a cause of disease in the developing fetus. The relationship between reproductive problem in dairy farm and positive herds (OR =3.6, P = 0.049) indicating that *T. gondii* might be one possibly cause of reproductive problem in small dairy herds in western Thailand. There is a report showed the isolation of viable *T. gondii* in 2 aborted bovine fetuses, 1 from the United States and 1 from Portugal. Both isolates were made by bioassay of fetal brains in mice (Canada, et al., 2002). It was indicated that *T. gondii* can be transplacentally transmitted in cattle.

Toxoplasmosis in dogs and cats

For Toxoplasmosis, of the 114 dogs and 36 cats sera sample tested, 7 sera samples from dogs (6.1%) and 3 sera samples from cats 8.3% were found the titer of *T. gondii* range from 1:64 to 1:256 in dogs and from 1:256 to 1:2048 in cats. The prevalence of dogs in dairy farm was lower than street dogs. Jittapalapong et al. (2007) report the prevalence

of *T. gondii* in stray dogs in Bangkok was 9.4% (40/427) and *T. gondii* antibodies were detected in 65 (11.0%) of the 592 cats.

In this study, the sex of dogs was not significantly associated with seroprevalence, as has been reported by other researchers (Dubey, 1985;Jittapalapong et al., 2007). No significant differences were observed in the seroprevalence of *T. gondii* in both the sexes of cats as similar to study in Japan (Maruyama et al., 2003).

For the seropositive in pets, the fecal transmission was the major cause of transmission by 4 times more likely to be positive in fecal contamination herds. Cats are the natural reservoir of *T. gondii* and excrete the resistant oocyst to environments. Therefore, cats should be not allow in farm areas and should keep in the house.

There was association between seropositive dogs and cats and positive dairy cows. In herd with positive pets in farms, the prevalence was 4 times more likely to have positive cows in herd and tend to associate with reproductive problems in dairy herds. Most dairy farms in the Western part Thailand had cats and dogs as pet and no boundary between dairy farm and their houses. Cats are capable of roaming all areas including the food storage and even in stalls. Therefore, the spreading of toxoplasmosis in dairy farms might rely on the number of dogs and cats roaming in the farm. The increasing number of pet will increase the risk of toxoplasmosis among animals in dairy farms.

In the positive household, the pregnancy abnormality of the owner was found 7.7 times more likely than negative household. The pregnancy abnormality of the owner was not investigated in the study. Women who become infected for the first time during pregnancy, and immunosuppressed patients are at serious risk from toxoplasmosis.

In human, *T. gondii* is transmitted either by direct animal contact, by ingesting oocysts, or by eating raw, undercooked meat or unpasteurised milk containing infective stages of the parasite. Consumption of infected meat or milk can facilitate zoonotic transmission. Tachyzoites of *T. gondii* have been found in the milk of several intermediate hosts, such as cat, sheep, goat and cows (Dubey, 1998; Tenter et al., 2000; Powell et al., 2001). High seroprevalence rates can occur among various groups of strict vegetarians (Hall et al., 1999), so transmission by oocysts may be more common than previously assumed.

In conclusions, our results showed that *T. gondii* infection was present in diary cattle and farm dogs and cats in western province. The risk factors associated with *T. gondii* infections were a present of cats in farms and relationship between reproductive problem in dairy farm and positive herds. The fecal contamination in feed was the major source of transmission in the study. *Toxoplasma gondii* is not only another protozoans that should be considered in the differential diagnosis of protozoal abortion in cattle, but also has implications for public health.

Table 1.
Seroprevalence of *T. gondii* and *N. caninum* classified in western provinces.

Province	Rachaburi		Nakhon Pathom		Kanchanaburi		Total	
	Herd	Individual	Herd	Individual	Herd	Individual	Herd	Individual
T. gondii	25%	9%	40%	9%	15%	3%	26.7%	7%
N. caninum	45%	13%	40%	10%	20%	6%	35%	9.7%

Table 2.
Results of Toxoplasmosis and Neosporosis disease status calssifeid in parity

Parity	Neosporosis		Toxoplasmosis	
	Positive (+)	Negative (-)	Positive (+)	Negative (-)
0	4	8	2	10
1	5	70	6	69
2	7	59	4	62
3	3	57	3	57
4	3	34	3	34
5	2	20	0	22
6	1	15	2	14
7	1	5	1	5
8	2	3	0	5
9	1	0	0	1

Table 3.
Unconditional association between disease status (+/-) and other measured variables using logistic regression with random herd effect in 300 dairy cows from 60 herds in western part Thailand**.**

Variables	Random Effects Regression (P-value)	
	Neosporosis	Toxoplasmosis
Province	0.311	0.234
Herd size	0.131	0.394
The present of dog	0.574	0.992
The present of cat	0.543	0.009
The present of mouse	0.069	0.791
The present of other animals	0.014	0.591
Abortion in farm	0.106	0.465
Reproductive problem	0.854	0.051
Barn type	0.153	0.946
Parity	0.720	0.609

Table 4.
Results from the multivariable logistic regression evaluating the relationship between neosporosis and risk factors in 300 cows from 60 herds in western part Thailand**.**

Variables	b	S.E.	P-value	OR	95% CI OR	
other	0.99	0.40	0.014	2.69	1.22	5.92
mouse	1.26	0.75	0.094	3.52	0.81	15.22

Table 5.
Results from the multivariable logistic regression evaluating the relationship between toxoplasmosis and risk factors in 300 cows from 60 herds in western part Thailand**.**

Variables	b	S.E.	P-value	OR	95% CI OR	
Present of cat	2.64	1.04	0.009	13.98	1.83	106.64
Reproductive problem	1.27	0.65	0.049	3.58	1.01	12.75

Table 6.
Seroprevalence of *T. gondii* in dogs and cats and *N. caninum* in dogs classified in western provinces.

Province	Rachaburi		Nakhon Pathom		Kanchanaburi		Total	
	dogs	cats	dogs	cats	dogs	cats	dogs	cats
Toxoplasma gondii	3	1	1	1	3	1	7	3
Neospora caninum	4	0	3	0	1	0	8	0

Table 7. Antibody levels of *T. gondii* in dogs and cats.

Species	No.tested	No. (%) of samples showing the antibody titer at					
		1:64	1:128	1:256	1:512	1:1024	1:2048
Dogs	114	1	1	5	0	0	0
Cats	36	0	0	1	1	0	1
Total	150	1	1	6	1	0	1

Table 8. Unconditional association between disease status (+/-) and other measured variables using logistic regression with random herd effect in 150 sera from 40 herds in western part Thailand**.**

Risk factors	Neosporosis		Toxoplasmosis	
	OR	P-value	OR	P-value
Sex	1.97	0.36	0.75	0.66
Age	9.30	0.04	1.88	0.34
Born in farm	2.23	0.39	1.07	0.92
Illness	1.73	0.46	0.69	0.61
Death	0.71	0.34	0.74	0.31
Feces contamination	0.43	0.26	4.61	0.06
Drink raw milk	0.81	0.80	1.73	0.62
Pregnant abnormality	0.00	1.0	7.67	0.10

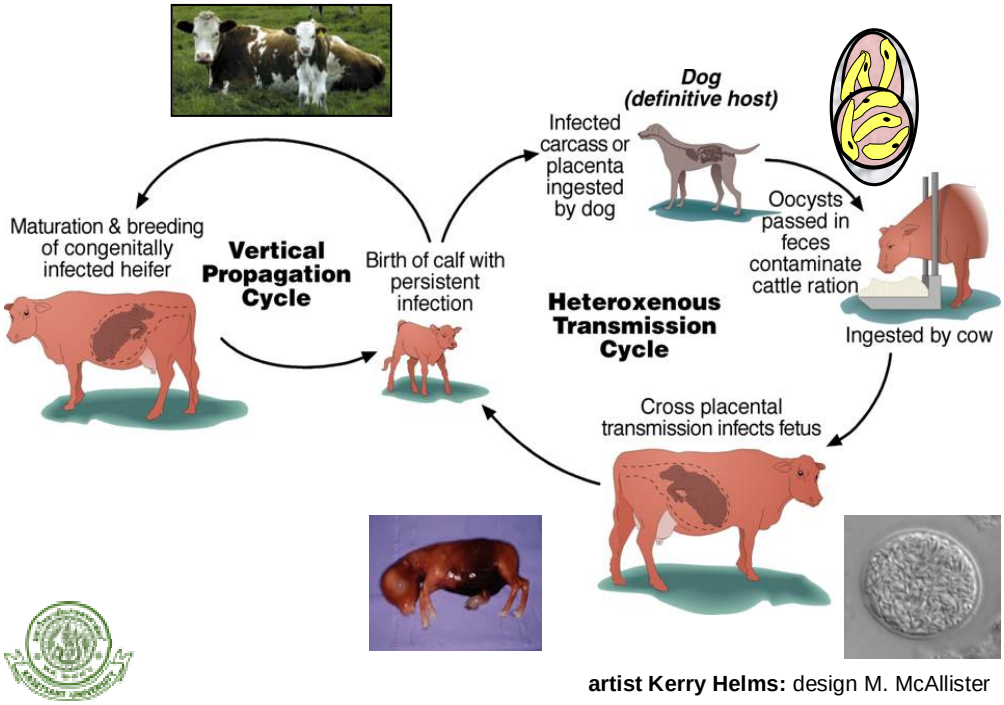

Figure 1. Transmission of *Neospora caninum* in Domestic Animal

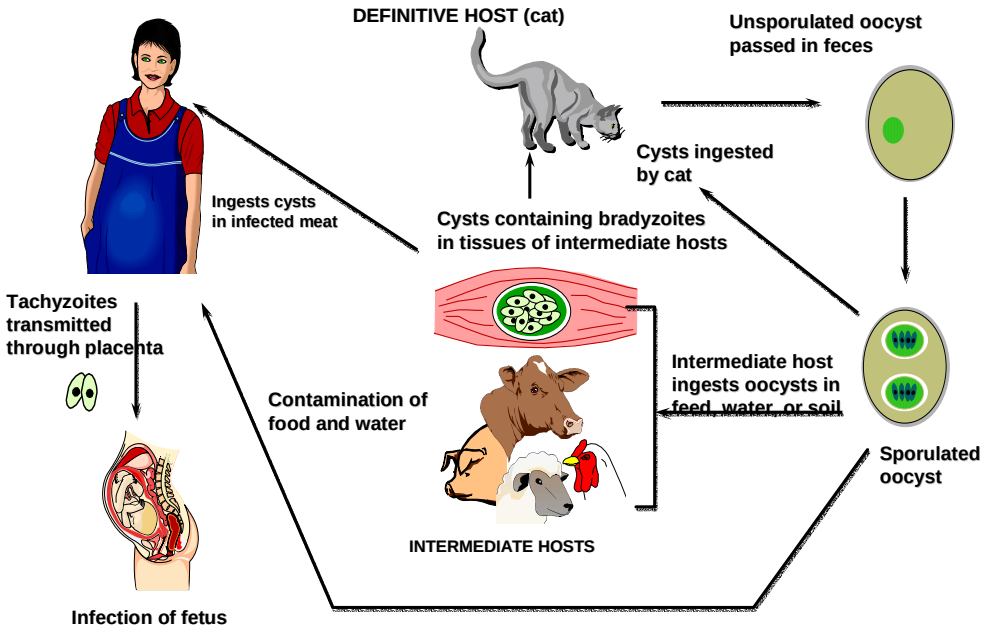

Figure 2. Transmission of *Toxoplasma gondii* in Animals and Human

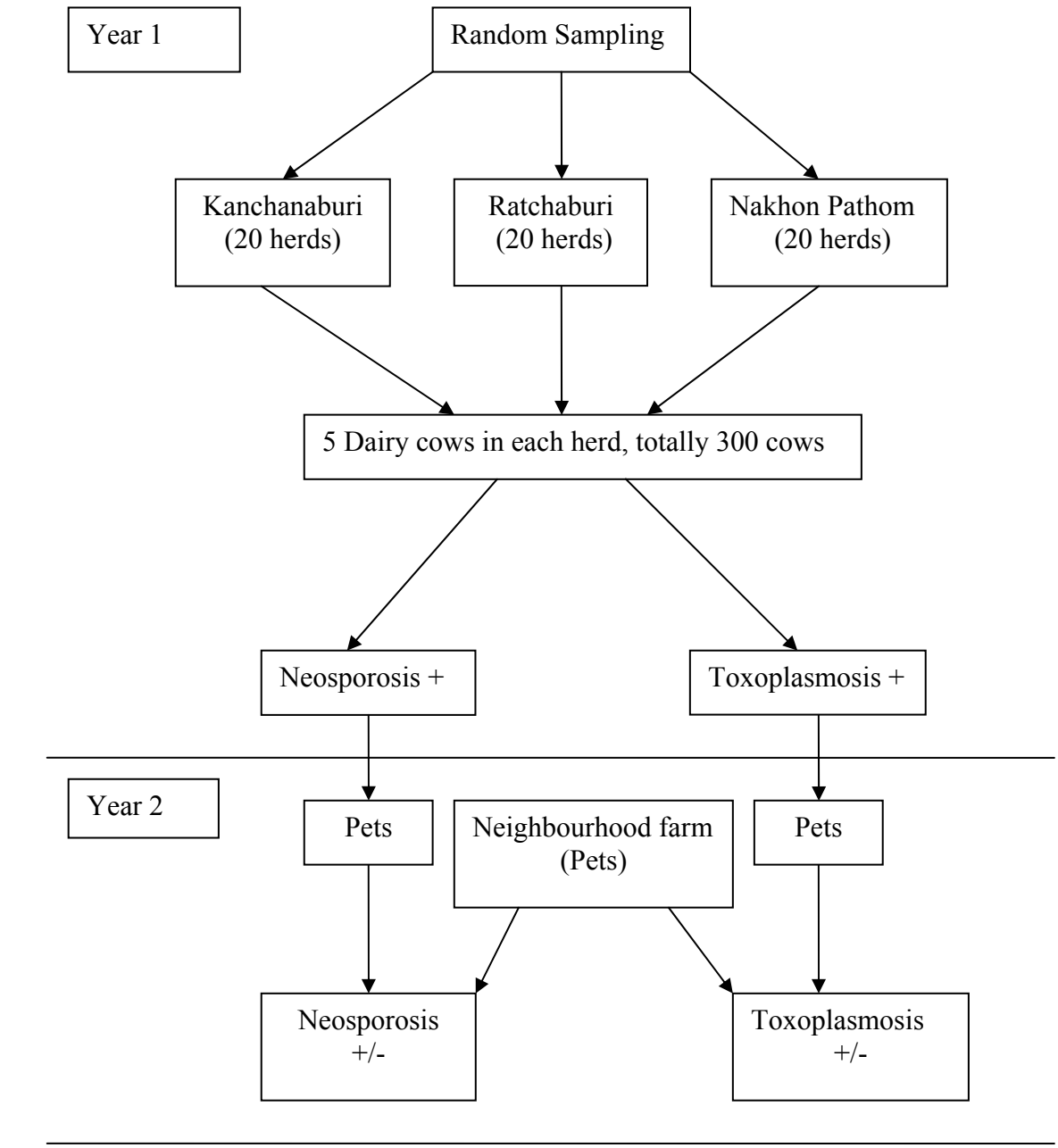

Fig 3. Research plan in 2 year periods.

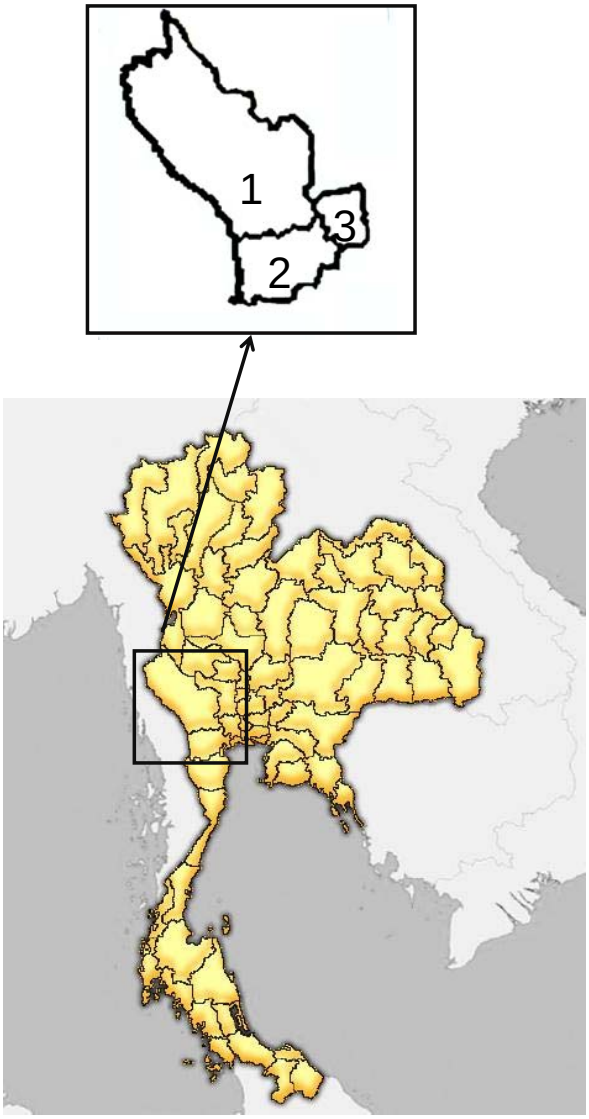

Figure 4. Map of Thailand (below) and western part (above). The numbers indicate the provinces as follows: 1.Kanchanaburi 2.Ratchaburi 3.Nakhon Pathom

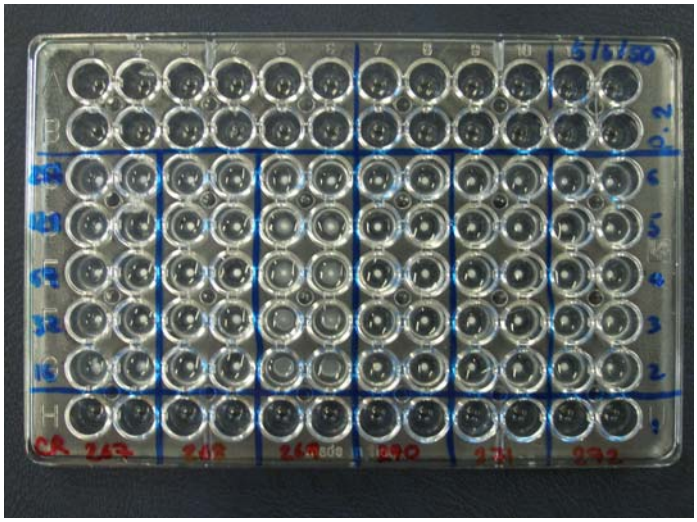

Figure 5. The positive and negative results of Latax agglutination.

Figure 6. The positive and negative results of C-ELISA.

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เอกสารแนบหมายเลข 3

Output จากโครงการวิจัยที่ได้รับทุนจาก สกว.

1. ผลงานตีพิมพ์ในวารสารวิชาการนานาชาติ (ระบุชื่อผู้แต่ง ชื่อเรื่อง ชื่อวารสาร ปี เล่มที่ เลขที่ และหน้า) หรือผลงานตามที่คาดไว้ในสัญญาโครงการ

Pipat Arunvipas, Tawin Inpunkaew, Sathaporn Jittapalapong**.** Risk factors and seroprevalence of antibodies to *Toxoplasma gondii* in dairy in Western Thailand. Preparation for submit to Veterinary Parasitology Journal.

Pipat Arunvipas, Tawin Inpunkaew, Sathaporn Jittapalapong**.** Risk factors and seroprevalence of antibodies to *Neospora caninum* in dairy in Western Thailand. Preparation for submit to Veterinary Parasitology Journal.

2. การนำผลงานวิจัยไปใช้ประโยชน์
   - เชิงวิชาการ (มีการพัฒนาการเรียนการสอน/สร้างนักวิจัยใหม่)

3. อื่นๆ (เช่น ผลงานตีพิมพ์ในวารสารวิชาการในประเทศ การเสนอผลงานในที่ประชุมวิชาการ หนังสือ การจดสิทธิบัตร)

Arunivipas, P., Maruyama, S. Jittapalapong, S. Seroprevalence of *Toxoplasma gondii* infections in dairy cows in Western Thailand. Proceeding of The 3rd Kasetsart University Kamphaengsaen (KU-KPS) Conference, 6-7 December, 2006. Nakhon Pathom, Thailand.

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