

Seroprevalence and Risk Factors of *Neospora caninum* Infection among Dairy Cows in the Western Provinces of Nakhon Pathom, Ratchaburi and Kanchanaburi, Thailand

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ABSTRACT

A total of 300 dairy cow sera from 60 dairy herds from three provinces (Nakhon Pathom, Ratchaburi and Kanchanaburi) in western Thailand were collected and examined for antibodies against *N. caninum* infection by competitive enzyme-linked immunosorbent assay and an indirect fluorescent antibody technique. Of these, 10% were positive to *N. caninum* infection and the herd prevalence was 35% (21/60). The presence of domestic dogs on the farms did not demonstrate a significant association with *N. caninum* infection in this study. Multiple logistic regression was used to evaluate the association between the risk variables collected from questionnaires and sero-positivity to *N. caninum* infection among dairy cows. A herd with the presence of backyard chickens was 2.7 times more likely to be seropositive than those herds without chickens ($P=0.01$) and herds with rats on the farms were 3.5 times more likely to get infected. Seropositive cows tended to have a higher risk of abortion than seronegative animals (odds ratio = 2.4, $P=0.08$) which suggested that *N. caninum* could potentially be a major cause of abortion among dairy cows in western Thailand.

Keywords: *Neospora caninum*, seroprevalence, dairy cows, risk factors, western Thailand

INTRODUCTION

Neosporosis has emerged as a serious disease of cattle and dogs worldwide (Dubey, 1999) and is a major cause of abortion in dairy cattle in the USA (Anderson *et al.*, 1995). *Neospora caninum* is an apicomplexan protozoa that was first recognized in dogs in Norway in 1984 (Bjerkas *et al.*, 1984). Its principal route of infection in cattle is via transplacental transmission (Dubey *et al.*, 1992), which is considered one of the most efficient means of transmission to cattle (Bjorkman *et al.*, 1996). Horizontal transmission

also occurs via *Neospora* sporulated oocysts in contaminated food and water (McAllister *et al.*, 1998). There is no direct transmission of *N. caninum* infection among cows in the same herd. *N. caninum* infected calves may show clinical signs such as neurologic symptoms, underweight, difficulty to rise, or may show no clinical signs (Dubey *et al.*, 2007). Seropositive cows remain infected for life (Pare *et al.*, 1996). Seropositive cows are more likely to abort than seronegative cows and this has an impact on both the dairy and beef cattle industries (Lopez-Gatius *et al.*, 2004). Moreover, abortion by neosporosis may repeatedly

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occur in these animals (Moen *et al.*, 1998). In Canada, milk production from seropositive cows was 250 to 300 kg less than from seronegative cows (Hobson *et al.*, 2002).

A high prevalence of neosporosis has been reported worldwide—in dairy cattle in the Netherland (Wouda *et al.*, 1999), water buffaloes in Egypt (Dubey *et al.*, 1998) and Vietnam (Huong *et al.*, 1998) and in cattle in Korea (Bae *et al.*, 2000), Vietnam (Huong *et al.*, 1998), and Thailand (Suteeraparp *et al.*, 1999; Kashiwazaki *et al.*, 2001; Chanlun *et al.*, 2002; Kyaw *et al.*, 2004). At present, there is no evidence of *N. caninum* infection in humans (McCann *et al.*, 2008).

In Thailand's northeastern region, using an indirect fluorescent antibody technique (IFAT), seroprevalences were found to range from 12 to 70% in dairy cattle (Suteeraparp *et al.*, 1999; Kashiwazaki *et al.*, 2001). Suteeraparp *et al.* (1999) reported seroprevalence of 6% in dairy cattle from central Thailand, while Chanlun *et al.* (2002) found 46% seroprevalence in bulk milk-tank samples.

Neospora seropositivity in cattle is associated with many risk factors, such as the presence of dogs and poultry on the farms and the feeding of moldy fodder. However, horizontal transmission of *N. caninum* within and between farms may be due to other factors. Thus, in order to control the disease at the farm level, it is important to determine factors which increase the probability of *N. caninum* infection. Limited information was available on the epidemiology of neosporosis in Thailand. Therefore, investigation of the epidemiological status of this disease was needed so that preventive and control measures could be implemented and monitored. Therefore, the purposes of this study were to assess the seroprevalence of *N. caninum* infection and to determine the risk factors of *N. caninum* among dairy cows in some western provinces of Thailand.

MATERIALS AND METHODS

Animals and blood collections

Sample collections were carried out in three western provinces of Thailand (Nakhon Pathom, Rachaburi, and Kanchanaburi; Figure 1). The study was carried out between June 2006 and September 2007. Most dairy farms in this



Figure 1 Map of Thailand showing provincial boundaries with inset showing the western provinces involved in the study. (1=Kanchanaburi; 2=Ratchaburi; 3=Nakhon Pathom).

area carry out intensive rearing of animals. The sample size ($n = 246$ animals) was calculated by considering a large population ($n = 100,000$), 20% prevalence, 95% confidence levels and 5% precision (error). Blood samples were collected from 20 dairy farms which were randomly selected in each province. A total of 60 herds were included in the study. Five cows from each herd were randomly selected and a total of 300 samples were included. Data were obtained by questionnaires regarding the state of herd management, breed, age and health condition. Most cows were cross bred Holstein Friesian belonging to small-holder farms ranging from 5 to 20 milking cows and the number of lactating cows ranged from 5 to 80. Most cows were tied in a stall barn with component feeding. The blood was collected by caudal venepuncture, centrifuged at $500 \times g$ for 10 min, separated and stored at -20°C until serological analysis.

Serological assay

Three hundred cow sera were tested for antibodies against *N. caninum* infection, using competitive enzyme-linked immunosorbent assay (c-ELISA; VMRD, USA). Briefly, 50 μL of serum samples were transferred onto an antigen-coated plate for each sample including negative and positive controls. Each plate was incubated at room temperature for 1 hr, then washed three times with washing buffer solution. Antibody-peroxidase conjugate (50 μL) was added to each well, incubated for 20 min at room temperature and washed three times with washing buffer solution. Then, 50 μL of substrate were added into each well and the plate was incubated for 20 min, after which stop solution was added. Finally, the plate was measured for optical density by a microplate reader (Tecan, Austria) at a wavelength of 630 nm. All positive samples were confirmed by IFAT.

For IFAT, antigen slides were incubated with cow sera diluted 1:100 in 5% bovine serum albumin (BSA) with phosphate buffer solution (PBS) for 1 hr at 37°C and then with a fluorescein

isothiocyanate-labeled goat anti-bovine IgG diluted 1:200 in 5% BSA with PBS. Positive and negative control sera were included in each slide. Slides were examined using a fluorescence microscope (Olympus, Japan) and only bright, linear peripheral fluorescence of trophozoites was considered to be positive.

Statistical analysis

Descriptive statistics were calculated and unconditional associations between disease status and each variable were determined. Ten variables were assessed using a chi-square test for unconditional association measurement with: presence of cats, dogs, rats and other animals; (backyard chickens) on the farms; herd size (small, 10–40 animals; large, > 40 animals); barn type (tie stall, free stall, both); parity (0–9); reproductive problems; abortions on the farm; and province. The variables having P -values < 0.15 from univariate analysis were analyzed with multiple logistic regression (backward stepwise) to identify risk factors associated with *Neospora caninum* infection. Goodness-of-fit of the final model was examined to assess how well the model fitted the observed data. All analyses were conducted using the statistical software package STATA (version 8.2, Stata Corp., College Station, TX).

RESULTS

Twenty nine cows (9.7 %) were seropositive for *N. caninum* infection. The percentages of seroprevalence of neosporiasis in dairy cows in Kanchanaburi, Nakhon Pathom and Ratchaburi were 6, 10, and 13%, respectively. Herd prevalence was 35% (21/60) and Ratchaburi province had the highest percentage of positive herds with 45% (9/20), followed by values of 40% (8/20) and 15% (3/20) in Nakhon Pathom and Kanchanaburi provinces, respectively. No significant difference was observed among the provinces. All positive results were found in all

lactations (1–9), including pregnant heifers (Table 1).

The presence of dogs or cats, reproductive problems on the farms, parity of cows and province did not show significant association in unconditional variables (Table 2). Herd size, the presence of rats and other animals (backyard chickens), history of abortion on the farm and

barn type were significant for the disease status in the unconditional analysis. Two variables were fitted in the final model. The presence of backyard chickens contributed significantly to *N. caninum* seropositive cows and the presence of rats on the farm had a tendency of horizontal transmission of *N. caninum* among the cattle (Table 3).

Table 1 Results of *Neospora caninum* infection classified in parity total of 29 positive and 271 negative samples out of 300.

| Parity | Neosporiasis (+) | Neosporiasis (-) |
|---------|------------------|------------------|
| Heifers | 4 | 8 |
| 1 | 5 | 70 |
| 2 | 7 | 59 |
| 3 | 3 | 57 |
| 4 | 3 | 34 |
| 5 | 2 | 20 |
| 6 | 1 | 15 |
| 7 | 1 | 5 |
| 8 | 2 | 3 |
| 9 | 1 | 0 |

Table 2 Unconditional association between infection status (+/-) and other measured variables using logistic regression (n = 300).

| Variable | OR | Random effects regression (<i>P</i> -value) |
|----------------------|-----|--|
| Province | 1.7 | 0.31 |
| Herd size | 2.9 | 0.13 |
| Presence of dogs | 1.7 | 0.57 |
| Presence of cats | 1.3 | 0.54 |
| Presence of rats | 4.3 | 0.07 |
| Presence of chickens | 3.2 | 0.01 |
| Abortion in farm | 2.4 | 0.08 |
| Reproductive problem | 0.9 | 0.85 |
| Barn type | 1.9 | 0.15 |
| Parity | 0.9 | 0.63 |

OR = Odds ratio.

Table 3 Multivariable logistic regression evaluating the relationship between Neosporiasis and risk factors.

| Variable | b | SE | P-value | OR | 95% CI OR |
|----------------------------------|------|------|---------|------|---------------|
| Other animals (backyard chicken) | 0.99 | 0.40 | 0.01 | 2.69 | [1.22, 5.92] |
| Rats | 0.26 | 0.75 | 0.09 | 3.52 | [0.81, 15.22] |

OR = Odds ratio; 95% CI OR = 95% level confidence limit interval for odds ratio.

DISCUSSION

In this investigation, 35% (21/60) of dairy herds had at least one seropositive animal to *N. caninum* infection indicating that *N. caninum* was widely infected among dairy herds in some of the western provinces of Thailand. Ratchaburi province had the highest proportion of seropositive cows with 13% (13/100) and herds with 45% (9/20). The overall prevalence of *N. caninum* infection was 9.7% (29/300). This result was similar to the previous study by Jittapalpong *et al.* (2008) which reported 11.7% seroprevalence of *N. caninum* infection among dairy cows in some north-east provinces of Thailand. However, the current result was higher than the previous serological survey (6%) in the central part of Thailand (Suteeraparp *et al.*, 1999). The increasing prevalence may be partially due to different diagnostic methods and the distribution of animal sampling. Moreover, Kyaw *et al.* (2004) who studied two sub-districts of Nakhon Pathom province found that individual and herd prevalences were 5.5% (30/549) and 34% (20/59), respectively, with the same diagnostic tests. When compared to the 10% (10/100) individual and 40% (8/20) herd prevalences in the current study, it is suggested that the extent of infection or exposure among the dairy population in Thailand is most likely to have increased. Positive results were seen in all lactations, including pregnant heifers; however, parity was not significant in the study.

It has been demonstrated that domestic dogs can act as a definitive host of *N. caninum* (Dubey and Lindsay, 1996). This was proved by McAllister *et al.* (1998) who demonstrated that dogs fed mouse tissues containing *N. caninum* tissue cysts were capable of shedding oocysts. Dogs are suspected to play an important role in horizontal transmission, particularly in point-source epidemic exposure of cattle to *N. caninum* (Bartels *et al.*, 1999; McAllister *et al.*, 2000). The presence and the number of dogs was correlated to

the high seroprevalence of *N. caninum* infection in dairy cattle (Paré *et al.*, 1996; Wouda *et al.*, 1999). However, the presence of dogs on the farms was not significant in the current study, agreeing with a previous study by Kyaw *et al.* (2004).

Seropositive cows tended to have a higher risk of abortion than seronegative animals (odds ratio (OR) = 2.4, $P = 0.08$), suggesting that *N. caninum* might be one of the causes of abortion among dairy cows in Thailand which was similar to Davidson *et al.* (1999) who found a strong relationship between seropositivity and abortion, 3.5 times more often than in seronegative cows (OR = 3.49, $P < 0.05$).

Herd size was a significant variable in the unconditional association analysis. Large-sized herds (more than 40 dairy cows) were three times more likely to have seropositive dairy cows. Dairy cows served as intermediate hosts that cannot produce oocysts. Therefore, direct transmission among cows is not possible and only vertical transmission could be the main source of neosporosis and may significantly contribute to persistence of infection in the herd (Pare *et al.*, 1996). Normally, better herd management on large farms results in cows in those herds having longer lifespans when compared to cows from small herds. Once cows are infected with *N. caninum* either by horizontal or vertical transmission, they will probably remain persistently infected (Anderson *et al.*, 1997). Therefore, herd size may be confounding other results in the study.

Cows fed in free-stall barns were twice as likely to be seropositive than cows fed in tie-stall barns. Some herds allowed cows to graze even though fresh grass is usually cut and carried to the cows during the rainy season, creating greater chances for contact with the oocysts than for cows that are normally kept in a barn.

Another way of horizontal transmission is from other animals (chickens and rats) roaming on the farm. Herds on farms having backyard chickens were 2.7 times more likely to be seropositive than

other herds on farms without backyard chickens ($P = 0.014$) and this result correlated with the findings of Bartels *et al.* (1999), suggesting that the presence of chickens on the farm is one of the risk factors associated with abortion in dairy herds in the Netherlands. These animals could serve as oocysts carriers so that chickens might play a role as intermediate hosts by which a dog may become infected (having eating an infected chicken). In the current study, rats were also potential carriers for horizontal transmission of *N.caninum*. The farms that had rats roaming, especially in the feeding area, were 3.5 times more likely to have a seropositive herd ($P=0.094$). Lindsay *et al.* (1999) presented evidence that dogs became infected by eating either mice brains containing *Neospora* tissue cysts or by eating excreted sporulated oocysts. Further research is needed to find out the relationship between the presence of dogs on dairy farms and the prevalence of *N. caninum* infection in cattle.

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