

## Seroprevalence of *Neospora caninum* Infections of Dairy Cows in the North-east of Thailand

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### ABSTRACT

Livestock development particularly in dairy cows have been hampered by low production including milk and growth rate due to many pathogens including *Neospora caninum*. *N. caninum*, an obligate intracellular protozoan parasite, is the causative agent of neosporosis recognized as a major cause of bovine abortion around the world and known to have a detrimental effect on bovine pregnancy and on milk production. Currently, there is no effective method for control of neosporosis since there is less information regarding current status of infections. The objective of this study was to investigate the seroprevalence of neosporosis in dairy cows of the Northeast of Thailand. In 2007, the sera of 445 cows of 55 small holder farms from the top three highest consensus of dairy farms in the Northeast provinces such as Khon Kaen, Udorn Thani, and Sakon Nakhon were collected. Antibodies to *N. caninum* were assayed by c-ELISA (VMRD, USA) and 52 (11.7 %) found seropositive. Cows with age more than 5 years had the highest seroprevalence (13 %) compared to age between of 1 and 5 years (11.5%) and less than age of 1 year (6.1%). Khon Kaen had the highest endemic area for *N. caninum* infections in dairy cows (12.9%). A total of dairy farm infections was 58.2% (32/55). The high incidence of *N. caninum* infections in cows indicated the risk of Thai farmers to realize and understand the problem of dairy cow's infertility in their farms. This result would be beneficial for controlling strategy of neosporosis of food animals in Thailand.

**Key words:** dairy cows, cELISA, seroprevalence, Thailand, *Neospora caninum*

### INTRODUCTION

Neosporosis is recognized as a major cause of bovine abortion around the world and is known to have a detrimental effect on bovine

pregnancy outcome and on milk production (Thurmond and Hietala, 1997). *Neospora caninum*, the causative agent of bovine and canine neosporosis, is a parasite that belongs to a unique group of the protozoa that exploit predator-prey

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relationships to facilitate transfer between their final hosts (Dubey and Lindsay, 1996). The consequences of *Neospora* infections in a pregnant animal can be abortion of the fetus or birth of a weak calf or birth of a clinically healthy but persistently infected calf (Thurmond *et al.*, 1997). Currently, there is no effective method or treatment to control neosporosis. Prevention and control strategies are relied on farm management that will be economically or practically possible to implement on dairies and beef cattle facilities. The actual prevalence of neosporosis in both cattle and dogs will be utilized to reduce the infected cases in farms.

Several diagnostic methods for bovine neosporosis have been developed. The enzyme-linked immunosorbent assay (ELISA) and the indirect immunofluorescent assay test (IFAT) are the current serological test used for diagnosis of *N. caninum* infections. The advantages of serological testing include the relative ease of serum collection and the lack of need for an active infection in order to detect past exposure to the organism in question. Disadvantages of serological testing include the possibility of false positive diagnosis due to cross-reactivity between infectious organisms and discerning between animals that are susceptible to disease from those that may have been exposed to the organism yet are presently protected from further infection. While the former disadvantage can be overcome through careful development and validation of the diagnostic reagents and technique, the latter is a biological phenomenon that will be extremely difficult to surmount (Dubey *et al.*, 1997).

Recent studies have revealed that currently available serodiagnostic assays for exposure to *N. caninum* will detect antibodies in both aborting and non-aborting cattle, and that none of these assay were able to predict susceptibility to disease (e.g., the probability of abortion) for individual animals. This corroborates the reported futility of a similar ELISA-based test

for the diagnosis of a very similar disease, toxoplasmosis, in humans. However, serological testing remains a valuable tool as an economical means of evaluating past exposure of host to various pathogens and parasites.

Demonstration of *Neospora* infections in dairy herd will reveal the factor that might be one of the influenced impact on livestock development in developing countries such as Thailand. Sustainable strategy to control of economic losses due to neosporosis is depend on the number of infected animals and their locations, available reservoir hosts and quality of farm management. This appears to be an achievable objective given in this project.

In Thailand, there were a few investigations of neosporosis in cattle with inconclusive results since the cost of damages were not truly estimated or represent the real losses of Thai farmers. Suteeraparp *et al.*, (1999) was the first report of neosporosis in dairy cattle in the central part of Thailand with the seroprevalence of 6%. From 2001 to 2007, there were variations of seroprevalence from 5.5 to 62.5% in dairy cattle from Central to Northeast areas of Thailand (Kashiwazaki *et al.*, 2001; Kyaw *et al.*, 2004; Chalun *et al.*, 2007). More information will be beneficial for reducing cost of animal owners.

The objective of this study was to determine the seroprevalence of *Neospora* infections among dairy cows of North-eastern provinces. This data will be beneficial for prevention and control for veterinarians work in the areas and will help save Thai farmer to reduce the cost of livestock production.

## MATERIAL AND METHOD

### Sample size

The study was carried out between March to September 2007. All dairy cows were bred for milk production and most dairy herds in

the study was a small farm holder ranging 5-20 milking cows and were tie stall barn with component feeding. A total of 445 Holstein-Friesian cows were randomly selected from 55 dairy farms in 3 provinces with the high consensus of dairy population from North-eastern part of Thailand (Khon Kaen, Udonthani and Sakon Nakhon province)(as shown in table 1). Proportionally sample base on number of farm, the district which has more than 50 farms were randomly selected. Four districts were selected from Khon Kaen province, while 3 districts were selected from Udon Thani province and 1 district was selected from Sakon Nakhon province, respectively. The number of dairy cows sampling varied depends on the number of the cow population in the farm ranging between 5-20 samples per farm. Blood was collected from the jugular vein or caudal vein and sera was separated after from blood cells. Sera were stored at -20°C until used. Ages were classified into 3 groups, less than 1 year, 1 to 5 year, and more than 5 year old, respectively.

### Serological method

The competitive enzyme-linked immunosorbent assay (c-ELISA) was used in this

study (VMRD, USA). 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 hour then washed 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 for 3 times. Fifty microliters 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 measured through the optical density at 630 nm immediately.

### RESULT

The overall prevalence of *Neospora caninum* was 11.7%. Cows aged more than 5 years had the highest seroprevalence (13 %) compared to between 1 and 5 years (11.5%) and less than 1 year (6.1%). A total of dairy farm infections were 58.2% (32/55). Khon Kaen was the highest endemic area for *N. caninum* infections in dairy cows (12.9%). However, Muang district (Udon Thani) was the highest area for *Neospora* infection (100 %) following by Si Thaj (Udon

**Table 1** Samples collected from three provinces in north-eastern part of Thailand.

Province	No. of cows population	No. of cows sampling	No. of farm population	No. of farm sampling
Khon Kaen	14,033	233	584	28
-Muang	7,025	110	254	11
-Ubolrattana	842	48	50	6
Nam Phong	2,850	25	125	4
Kra Nuan	2,693	50	107	7
Udon Thani	5,737	121	320	13
-Muang	527	15	41	3
-Si That	2,542	76	115	5
Kut Chap	868	30	55	5
Sakon Nakhon	4,935	91	355	14
-Muang	1,425	91	127	14
<b>Total</b>	<b>24,705</b>	<b>445</b>	<b>1,259</b>	<b>55</b>

**Table 2** Factors affecting associated with *Neospora caninum* infections of dairy cows in the North-east of Thailand.

Factors	Category	Number of examined	Number of positive (%)	
Age	Less than 1 year	33	2(6.1)	
	1-5 year	227	26(11.5)	
	>5 years	185	24(13)	
Farm holders	<b>Khon Kaen</b>	<b>28</b>	<b>16(57.1)</b>	
	-Muang	11	7(63.6)	
	-Ubolratana	6	2(33.3)	
	-Nam Phong	7	5(71.4)	
	-Kra Nuan	4	2(50)	
	<b>Udon Thani</b>	<b>13</b>	<b>7(53.9)</b>	
	-Muang	3	3(100)	
	-Si That	5	4(80)	
	-Kut Chap	5	0(0)	
	<b>Sakon Nakhon</b>	<b>14</b>	<b>9(64.3)</b>	
	-Muang	14	9(64.3)	
	Dairy cows	<b>Khon Kaen</b>	<b>233</b>	<b>30(12.9)</b>
		-Muang	110	15(13.6)
-Ubolratana		48	3(6.3)	
-Nam Phong		50	9(18)	
-Kra Nuan		25	3(12)	
<b>Udon Thani</b>		<b>121</b>	<b>11(9.1)</b>	
-Muang		15	4(23.7)	
-Si That		76	7(9.2)	
-Kut Chap		30	0(0)	
<b>Sakon Nakhon</b>		<b>91</b>	<b>11(12)</b>	
-Muang		91	11(12)	
Total			445	52(11.7)

Thani) (80%), and Nam Phong (Khon Kaen) (71.4%) respectively (as shown in table 2).

## DISCUSSION

The present study revealed that the seroprevalence of neosporosis in dairy herds in North-east of Thailand average 11.7%, which was close to 10-13% by the investigation of Chanlun *et al.* (2007). However, it was higher than the previous serological surveys (6%) by Suteeraparp *et al.* (1999) and 5.5% by Kyaw *et al.* (2004). Chanlun *et al.* (2002) reported that 81.8% of the

herds was seropositive, having a within herd seroprevalence range of 0-46%, in the northeast of Thailand. In this study, we found that the herd prevalence of dairy cows in the northeast was 58.2% compared to the herd prevalence in Europe varied tremendously from 16 to 76% (Bartels *et al.*, 2006). This prevalence results indicated the infertility of dairy farm in the North-eastern provinces of Thailand. No treatment or successfully elimination was reported recently; therefore, the screening tests were the only way to isolate negative animals from the positives.

Neosporosis in cattle is associated with many risk factors, such as the presence on the farm of dogs, cats, poultry, duck, pigeon, rabbits (Bartels *et al.*, 1999; McGuire *et al.*, 1999; Ould-Amrouche *et al.*, 1999). The presence of dogs on a farm has been a potential risk to provide the increasing chance of horizontal transmission through the ingestion of oocysts, shed by infected dogs. In this study, most farm had at least one dog in nearby environment.

Farm management has to get more strict on many precaution regarding rule and regulation in farm standard. For examples, no pet such as dogs allow in the dairy farm zone and this will help isolate animals from threatening pathogens. Screening diseases before moving animals in and out of farm will help decreasing the chance to introduce pathogen into the farm since livestock development was hampered by many pathogens including *Neospora*.

The high incidence of *N. caninum* infections in dairy cows indicated the risk of Thai farmers to realize and understand the problem of dairy cow's infertility in their farms. This result will be beneficial for control strategy of neosporosis of food animals in Thailand.

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