

Seroprevalence of *Brucella abortus*, *Neospora caninum*, and *Toxoplasma gondii* Infections of Dairy Cows in the South of Thailand

S. Jittapalpong^{1*}, N. Pinyopanuwat¹, W. Chimnoi¹, C. Kengradomkij¹, P. Arunvipas², N. Sarataphan³, S. aruyama⁴, M. Desquesnes⁵

¹Parasitology, ²Large Animal and Wildlife Clinical Science, Faculty of Veterinary Medicine, Kasetsart University, Thailand

³Bureau of Biotechnology in Livestock Production, Department of Livestock Development, Ministry of Agriculture, Pathumthani, 12000, Thailand

⁴Laboratory of Veterinary Public Health, College of Bioresource Sciences, Nihon University, Kanagawa, Japan

⁵Centre de Coopération Internationale en Recherche Agricole pour le Développement (CIRAD), Bangkok, 10900, Thailand ; CIRAD, UR Trypanosomes, Montpellier, F-34000 France

*Corresponding author

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Introduction

Brucellosis in cattle is usually caused by biovars of *Brucella abortus*. The disease is usually asymptomatic in nonpregnant females. Following infection with *B. abortus* pregnant adult females develop a placentitis usually resulting in abortion between the fifth and ninth month of pregnancy. Laboratory bio-safety manual of the World Health Organization (1) have classified *Brucella* in risk group III. Brucellosis is readily transmissible to humans, causing acute signs which may progress to a more chronic form and can also produce serious complications. Infection is often due to occupational exposure and is essentially acquired by the oral, respiratory, or conjunctival routes, but ingestion of dairy products constitutes the main risk to the general public (2).

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 (3). *Neospora caninum* is the causative agent of bovine and canine neosporosis. 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 (4). Prevention and control strategies are relied on farm management that will be economically or practically possible to implement on dairy and beef cattle facilities. The actual prevalence of neosporosis in both cattle and dogs will be utilized to reduce the infected cases in farms. In Thailand, there were a few reports of neosporosis in cattle (5) with inconclusive results since the cost of damages were not truly estimated or represented the real losses of Thai farmers. More information will be beneficial for reducing cost of animal owners.

Toxoplasmosis is a zoonotic parasitic disease with a worldwide distribution. It is capable of infecting all warm-blooded animals and is estimated to infect 4 to 77 % of human population (6). Although not normally a significant problem for healthy individuals, *Toxoplasma* infection can be life threatening for congenitally infected and pharmacologically- immunosuppressed patients (7).

However, its greatest impact is in late AIDS, where up to 25% of patients will develop toxoplasmic encephalitis (8-9). In humans, *T. gondii* is transmitted either by ingesting oocysts or by eating raw or undercooked meat or unpasteurised milk containing infective stages of the parasite (10-11). While *T. gondii* can be transmitted directly by animal-human contact or through contact with contaminated feces, soil and herbage, it can also be transmitted through contaminated food and water. In animals, infection does not only result 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. Demonstration of *Brucella*, *Neospora* and *Toxoplasma* infections in dairy herd will reveal the factor that might be one of the influenced impact factor to livestock development in developing countries such as Thailand. Sustainable strategy to control economic losses due to these diseases depends 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. The objective of this study is to determine the seroprevalence of *Brucella*, *Neospora* and *Toxoplasma* infections among dairy cows of the Southern provinces. This data will be beneficial for prevention and control for veterinarians working in the areas and will help Thai farmers to reduce the cost of livestock production.

Materials and Methods

Sample size: A total of 187 Holstein-Friesian cows were randomly selected from 14 dairy farms in a province from the southern part of Thailand during 2007. Blood was collected from the jugular vein or caudal vein and serum was separated from blood cells. Sera were stored at -20°C until used. Ages were classified into 3 groups, 0 to 1 year old, 1 to 5 year old and more than 5 year old respectively.

Serological method: The competitive enzyme-linked immunosorbent assay (c-ELISA, VMRD, USA) was used to detect antibodies against *N. caninum* in this study. Detection of serum antibodies to *Brucella abortus* and *B. melitensis* were performed in this

study (c-ELISA, SAVONOVIR, Sweden). The presence of *T. gondii* antibodies was analyzed by the latex agglutination test kit (Eiken, Japan) and followed the protocol of Tsubota et al. (12-13). The procedure described in a previous report (14) was followed accurately. According to the kit instructions, the cut-off titer for this test was at 1:64. The results obtained were analyzed by the chi-square test, and the level of significance was set at $p < 0.05$.

Results and Discussion

Table 1 summarizes the results. 5.4% of the samples (10/186) were seropositive to *N. caninum* while no sample was seropositive to *B. abortus*. 12.3% of the samples (23/186) were seropositive to *T. gondii*. In *Neospora* infections, cows less than 1 year had the highest seroprevalence rate (10%) compared to more than 5 years (6.3%) and between 1 and 5 years (4.5%). In *Toxoplasma* infections, cows more than 5 years had the highest seroprevalence rate (10.9%) compared to less than 1 year (10%) and between 1-5 years (4.8%). A total of dairy farm infected by *Neospora* and *Toxoplasma* was 57.1% (8/14) and 71.4% (11/14), respectively.

No seropositive *B. abortus* in cows in the South indicated that brucellosis infection was properly controlled by prophylaxis campaigns. In fact, a routine diagnosis of brucellosis is mandated for animal owners to pass the test once a year since brucellosis screening test is regulated by law. Therefore, most farmers are educated and experience for this disease.

The present study revealed that the seroprevalence of neosporosis in individual dairy cattle in the South of Thailand average 5.4%, which was close to the previous serological surveys (6%) in Thailand (5). No treatment or successful elimination was reported recently; therefore, the screening tests were the only way to isolate negative animals from the positives. The high incidence of *N. caninum* (57.1%) and *T. gondii* (71.4%) infections in dairy herds stressed the risk for dairy cow's infertility and also for Thai farmers. Veterinarians and farmers should then build together prophylaxis program to eliminate the disease and reduce the risk for human contamination.

The South of Thailand has a long rainy season (6 months), which may be suitable for oocyst survival. Furthermore, cats are common pets among Islamic people. An increasing cat population may have relation to possibilities of infection from definitive hosts for the parasite. Most dairy farms in the South had cats 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. Cats play an important role in human toxoplasmosis (16), but dairy cows could form a link in the chain of transmission of this disease to human through the vehicles of raw milk and meat (17).

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References

- 1 WHO, 2006.: 1-89.
- 2 Bricker, 2002. Vet. Microbiol. 90: 433-434.
- 3 Thurmond and Hietala, 1997. Am. J. Vet. Res. 58: 1381-1385.
- 4 Thurmond et al., 1997. J. Vet. Diagn. Invest. 9: 44-49.
- 5 Suteeraparp et al., 1999. Vet. Parasitol. 86: 49-57.
- 6 Tenter et al., 2000. Int. J. Parasitol. 30: 1217-1258.
- 7 Chintana et al., 1998. Southeast Asian J. Trop. Med. Public Health. 29: 383-386.
- 8 Luft et al., 1984. J. Am. Med. Assoc. 252: 913-917.
- 9 Lucas et al., 1993. AIDS. 7: 1569-1579.
- 10 Riemann et al., 1975. J. Pediatr. 87: 1728-1732.
- 11 Sacks et al., 1982. J. Am. Vet. Med. Assoc. 148: 1728-1732.
- 12 Tsubota et al., 1977a. Jpn. J. Parasitol. 26: 286-290.
- 13 Tsubota et al., 1977b. Jpn. J. Vet. Sci. 26: 291-298.
- 14 Maruyama et al., 2003. Microbiol. Immunol. 47: 147-153.
- 16 Dubey et al., 1997. J. Parasitol. 83:1063-1069
- 17 Garcia et al., 1993. Acta Parastol. Portuguesa. 1: 332.

Table 1. Factors associated with *Brucella*, *Neospora* and *Toxoplasma* infections of dairy cows in the South of Thailand

Factors	Range	Sero-prevalence of <i>Neospora</i> infections (%)	Sero-prevalence of <i>Toxoplasma</i> infections (%)	Sero-prevalence of <i>Brucella</i> infections
Age	0-1 year	1/10 (10)	1/10 (10)	0
	1-5 year	5/112 (4.5)	15/112 (4.8)	0
	>5 years	4/64 (6.3)	7/64 (10.9)	0
Farm holders		8/14 (57.1)	11/14 (71.4)	0
Total		10/186 (5.4)	23/186 (12.4)	0