# Current Status of Brucellosis in Dairy Cows of Chiang Rai Province, Thailand

**Sathaporn Jittapalapong,[1]\* Tawin Inpankaew,[1] Arkom Sangwaranond,[1] Chamnonjit Phasuk,[1] Nongnuch Pinyopanuwat,[1] Wissanuwat Chimnoi,[1] Chanya Kengradomkij,[1] Chainirund Sununta[2] and Pipat Arunwipat[3]**

## ABSTRACT

Current status of brucellosis in dairy cows have an impact on livestock development in Chiang Rai due to transmission and creating infertile cows. Livestock development particularly in dairy cows have been hampered by low production including milk and meat production due to many pathogens including *Brucella abortus*, a gram negative bacteria, is the causative agent of brucellosis recognized as a major cause of bovine abortion around the world and known to have a detrimental effect on bovine pregnancy outcome and on milk production. Currently, there is no effective method for control of brucellosis. The objective of this study was to investigate the seroprevalence of brucellosis in dairy cows in Chiang Rai, Thailand. From January to June in 2007, the sera of 392 cows in 29 small holder farms from Chiang Rai were collected. Antibodies to *B. abortus* were determined by c-ELISA (SVANOVIR, Sweden) and 13 (3.3 %) samples were revealed seropositive. Cows between age of 1 and 5 years had the highest seroprevalence (3.8%) compared to cows with more than 5 years (2.8%). Phan district was the highest endemic area for *B. abortus* infections in dairy cows (60%). A total of dairy farm infections was 24.1% (7/29). The prevalence of individual *B. abortus* infections on cows was not high but the number of positive farms were rising. This result indicated the factor associated with dairy cow's infertility in dairy farms. This will be beneficial for control strategy of brucellosis such food safety program of food animals in Chiang Rai.

**Key words:** Brucellosis, dairy cow, Chiang Rai

## INTRODUCTION

Brucellosis is a world-wide zoonoses leading to great economic losses in the ruminant production system. Brucellosis in cattle is usually caused by biovars of *Brucella abortus*. In some countries, particularly in Southern Europe and Western Asia, where cattle are kept in close association with sheep or goats, infection can also be caused by *B. melitensis*. The disease is usually asymptomatic in non-pregnant females. Following infection with *B. abortus* or *B. melitensis*, pregnant

---

[1]  Department of Parasitology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok. 10900, Thailand.

[2]  Chiang Rai Provincial Office, Department of Livestock Development, Chiang Rai.

[3]   Department of Animal Resources and Exotic Animal, Faculty of Veterinary Medicine, Kasetsart University, Kamphaeng Saen. Nakhon Pathom 73140, Thailand.

\*  Corresponding author, e-mail: fvetspj@ku.ac.th.

adult females develop a placentitis usually resulting in abortion between the fifth and ninth month of pregnancy. Even in the absence of abortion, profuse excretion of the organism occurs in the placenta, fetal fluids and vaginal discharges (Nielsen, 2002).

Laboratory bio-safety manual of the World Health Organization (WHO) have classified *Brucella* in risk group III. Brucellosis is readily transmissible to humans, causing acute febrile illness – undulant fever – which may progress to a more chronic form and can also produce serious complications affecting the musculo–skeletal, cardiovascular, and central nervous systems. 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. There is an occupational risk to veterinarians and farmers who handle infected animals and aborted fetuses or placentae. Brucellosis is one of the most easily acquired laboratory infections, and strict safety precautions should be observed when handling cultures and heavily infected samples, such as products of abortion.

Genetic and immunological evidence indicates that all members of the *Brucella* genus are closely related and it has been proposed that the genus contains a single species of which the classical species (abortus, melitensis, etc.) would be more biovars. Nevertheless, there are real differences in host preference and epidemiology displayed by the major variants, as well as, molecular evidence of genomic variation (Nielsen *et al.,* 1995; Bricker, 2002).

The objective of this study is to investigate the prevalence of *Brucella* infection distributed among dairy cows in Chiang Rai province since this will help official veterinarians to understand the situation of this disease.

## MATERIAL AND METHOD

### Sample size

A total of 392 Holstein-Friesian cows were randomly selected from 29 dairy farms in Chiang Rai province. Blood was collected from the jugular vein or caudal vein and separated for serum after sedimentation of blood cells. Sera were stored at -20°C until used. Age was classified into 2 groups which is dairy cow 1 to 5 years old and more than five years old.

### Serological method

The competitive enzyme-linked immunosorbent assay (c-ELISA) for detection of serum antibodies to *Brucella abortus* and *B. melitensis* were performed in this study (SAVONOVIR, Sweden). Five microlitres of serum samples were diluted with 45 µl of sample dilution buffer and transferred into antigen-coated plate for each sample including negative, weak positive and positive control then added 50 µl of mAB-solution into all wells. The plate was incubated at room temperature for 30 minutes, then rinse with PBS-Tween buffer 4 times. Goat anti-mouse IgG horse-radish peroxidase conjugate (100 µl) were added to each well and incubated for 30 minutes at room temperature then washed with PBS-Tween buffer solution 4 times. One hundred microlitres of substrate solution were added into the well and incubated for 10 minutes then stop the reaction by adding 50 µl of stop solution for each well. The plate was measured the optical density at 450 nm within 15 minutes to prevent fluctuation in OD-values.

## RESULT AND DISCUSSION

The overall prevalence of *Brucella abortus* infection in this study was 3.3%. Cows aged between 1 and 5 years had the highest seroprevalence (3.8%) compared to more than 5

**Table 1**  Factors associated with *Brucella* infections of dairy cows in Chiang Rai

| Factors | Category | Number of examined | Number of positive (%) |
|---|---|---|---|
| Age | 1-5 year | 212 | 8(3.8) |
| | >5 years | 180 | 5(2.8) |
| Dairy farms | Muang | 4 | 1(25) |
| | Mae Lao | 5 | 0(0) |
| | Phan | 5 | 3(60) |
| | Thoeng | 9 | 1(11.1) |
| | Pha Ya Meng Rai | 2 | 0(0) |
| | Khun Tan | 3 | 1(33.3) |
| | Mae Sai | 1 | 1(100) |
| | total | 29 | 7(24.1) |
| Dairy cows | Muang | 80 | 4(5) |
| | Mae Lao | 30 | 0(0) |
| | Phan | 65 | 3(4.6) |
| | Thoeng | 100 | 1(1) |
| | Pha Ya Meng Rai | 28 | 0(0) |
| | Khun Tan | 37 | 1(2.7) |
| | Mae Sai | 52 | 4(7.7) |
| Total | | 392 | 13(3.3) |

years (2.8%). Phan district was the highest endemic area for *brucellosis* in dairy cows (60%) following by Khun Tan (33.3%), Muang (25%) and Thoeng (11.1%). A total of infected dairy farms was 24.1% (7/29).

The definitive diagnosis of brucellosis is made by the methods of culture and isolation procedures (WHO, 2006). However, it is not practicable when large number of animals are involved. Indirect ELISA was developed and used as the most specific serological test for brucellosis. Competitive ELISA has been developed to reduce the impact of vaccinal antibody and antibody derived from exposure to cross-reacting antigens on the serological diagnosis of brucellosis (Nielsen *et al.*, 2007).

The overall prevalence of brucellosis in individual animals in this study was not high (3.3%) as expected. However, the overall of herd infected by *Brucella* was rising (24.1%) due to the poor management or the higher frequency of detection. The high incidence of *B. abortus* infections in cows indicated that the Thai farmers do not realize and understand the problem of dairy cow's infertility in their farms. The management was based on the introduction of new animals to the farm without keeping animals in quarantine. Routine diagnosis of brucellosis associated with herd health management would be mandatory in the dairy farm since this will have the impact on farm production.

**ACKNOWLEDGMENTS**

## LITERATURE CITED

Bricker, B. 2002. Diagnosis strategies used for the identification of *Brucella*. **Veterinary Microbiology** 90: 433-434.

Nielsen, K., L. Kelly, D. Gall, P. Nicoletti and W. Kelly, 1995. Improved competitive enzyme immunoassay for the diagnosis of bovine brucellosis. **Veterinary Immunology and Immunopathology** 46: 285-291

Nielsen, K. 2002. Diagnosis of brucellosis by serology. **Veterinary Microbiology** 90: 447-459.

Nielsen, K., P. Smith, W. L. Yu, C. Elmgren, P. Nicoletti, B. Perez, R. Bermudez and T. Renteria, 2007. Second generation competitive enzyme immunoassay for detection of bovine antibody to *Brucella abortus*. **Veterinary Microbiology** 124: 173-177

WHO, 2006. Brucellosis in humans and animals. WHO (World Health Organization) in collaboration with FAO (Food and Agriculture Organization) and OIE (World Organization for Animal Health), M. J. Corbel (principal author). 89p