Factors Associated with Seropositive Antibodies to *Brucella melitensis* in the Nakhon Nayok, Thailand

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Backgroud: *Human brucellosis is a re-emerging disease in Thailand. In 2006, Her Royal Highness Princess Maha Chakri Sirindhorn Medical Center, Nakorn Nayok province had diagnosed three cases of brucellosis which have never been reported in this area.*
Objective: *To conduct an epidemiologic study with the aim of evaluating the sero-prevalence and factors associated with seropositive antibodies to Brucella melitensis among residents who live in the same sub-district of the first index case.*
Material and Method: *In 2007, a study was conducted in Chumpon sub-district, Ongkharak district, Nakhon Nayok province, Thailand where the outbreak took place in the previous year. The 86 subjects were selected from three villages in the present study area. Blood specimens were collected and tested for antibodies for Brucella melitensis using a serum agglutination test. A structural interview questionnaire was used to detect any possible risk factors. A binary logistic regression was utilized for analyzing the statistical data.*
Results: *Of all participants in the present study, 45.35% (95% CI; 34.61-56.08%) had seropositive antibodies to Brucella melitensis. Multivariate analysis indicated that factors associated with seropositive titers were highly related to contact with labored or aborted goats, adjusted odds ratio = 27.16 (95% CI = 1.02-721.53) and the consumption of raw goat products, adjusted odds ratio = 6.27 (95% CI = 1.25-31.36).*
Conclusion: *High seropositive prevalence of Brucella melitenis after the 2006 outbreak was found in the present study. The associated factors of infection are direct contact with infected animals and this is similar with the other outbreak areas in Thailand and the endemic countries. Therefore, local authorities should not only provide the communities with health education, but also conduct continued surveillance in order to help control and prevent the epidemic.*

Keywords: *Brucella melitensis, Seroprevalence, Zoonosis*

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Human brucellosis was first discovered in military personnel and reported by Bruce and et al in 1887[1]. This disease was recognized as zoonosis. The reservoir was livestock. Brucellosis was caused by *Brucella spp*., which was an aerobic, small, gram-negative coccobacilli. It was a facultative, intracellular pathogen that can persist in the environment invariably depending on temperature, pH, and humidity. Worse, *Brucella spp* could persist indefinitely if frozen or protected in aborted fetuses or placentas. There were more than 500,000 new cases reported annually worldwide. In a recently-updated global map; Mediterranean countries of Europe, North and East Africa, the Middle East, South and Central Asia and Central and South America were considered the endemic areas. Thailand is located in Southeast Asia and is considered in the group of non-endemic areas[2]. Brucellosis is an uncommon disease when compared with other zoonotic diseases in Thailand.

The first Thai brucellosis case was reported as early as 1970 in the Rayong. No cases have ever been reported prior to the current decade. Due to the government launched agricultural policy that promotes goat farming due to the fact that goat milk has higher protein content when compared to the milk from other animals. However, the success of this campaign has resulted with an accompanying animal brucellosis. The number of cases has gradually increased in Thailand.

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In recent years, there have been frequent outbreaks of this disease in the various provinces of Kanchanaburi, Satun; Phetchabun etc[3]. The Ministry of Public Health classifies this disease as a re-emerging disease in humans. Most of them were of a *Brucella melitensis* strain contracted from contact with goats or cattle[3,4]. However, there have never been reports of this disease in the Nakhon Nayok province.

In 2006, Her Royal Highness Princess Maha Chakri Sirindhorn Medical Center (HRH MSMC), Faculty of Medicine, Srinakharinwirot University had diagnosed three cases of brucellosis. According to epidemiological investigation, two of the three cases had been infected from a community that was exposed to goats. The first case was a 66-year-old female who lived near a goat farm and used goat faces as fertilizer. She presented with pancreatic abscess and had spinal bone involvement. The second case was a 71-year-old female, diagnosed four weeks after the first case. She came in with a fever of unknown origin and had a positive blood culture to *Brucella melitensis*. A few weeks later, the third case appeared. He was a 32-year old male laboratory technician in our hospital who presented with fever, myalgias and fatigue. He had blood cultures that grew the same species of the previous two cases. It was assumed that his infection was due to inadequate precautions taken during his work[5].

This incident has shown a lack of knowledge and insufficient guidelines for dealing with brucellosis. The epidemiological study was important data in the community health aspect in exploring risk factors related to contracting brucellosis. Public health personnel are in a position to take precautions to reduce these health risks and to protect the local peoples' health in the future. Therefore, the objective of the present study is to conduct an epidemiological study to evaluate the sero-prevalence and factors associated to seropositive antibodies to the *Brucella melitensis* infection. This is the same strain identified in the previous outbreak event among residents living in the same sub-district of the first index case. The present study is important for certain cases in the control and prevention of the spread of this disease in the community.

Material and Method

Study area

Nakhon Nayok province is located in the central region of Thailand, at the Northeast section of Bangkok, the capital city of Thailand and consists of 4 districts. One of the districts is the Ongkharak district where HRH MSMC is located. It consists of 11 sub-districts of 116 villages. The study area was the sub-district of Chumphon which is located 10 kilometers south of the Ongkharak district. (Fig. 1) It is comprised of 7 villages with a population of 4,613 residents. The major occupation of this area is agricultural; rice planting, goats and cattle farming. Most of the residents are Thai-Muslim and the majority of them are in the median income group.

An investigation was conducted in 2007 by a team from the Occupational Health Unit, Department of Preventive and Social Medicine, Faculty of Medicine, Srinakharinwirot University. During a community survey, the investigators found that there was an earlier brucellosis infection in one goat farm prior to the diagnoses of the three cases of brucellosis who are aforementioned report. The solution to this problem was to eradicate brucellosis from the goats to control further events. The present study team selected 3 villages of the seven and based the selection of the villages on the potential risk of infection. The first village is located on the west side of the sub-district where the first brucellosis case emerged as an index case. The second village is located in the central part of the sub-district, where there are goats and cattle husbandry. The third village is located on the north side of the sub-district, where there are no goats or cattle. The investigating team had cultured soil from the farm where the outbreak originated. Unfortunately, no growth of *Brucella spp.* was found. It is possible that the soil had been kept far too long for the *Brucella spp.* to

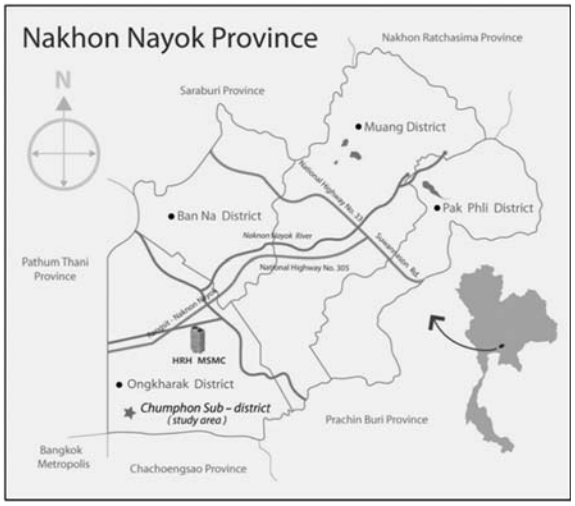

Fig. 1 Schematic map of Nakhon Nayok Province showing location of study area

survive[6].

Study samples and collection

The cross sectional study was approved by the ethics committee of the Faculty of Medicine, Srinakharinwirot University. Ninety residents from three villages volunteered to participate in the present study. Four of the volunteers refused to participate in blood collection. The present study excluded those cases, because blood testing was a requirement for the outcomes of the present study. When the informed consent form had been completed, each resident was interviewed in regard to their medical history. Potential risks such as; history of contact with goats and cattle, contact with the animals carcasses or secretions, the consumption of raw goat products, activities related to animal farming, knowledge about brucellosis and personal hygiene were anecdotally acquired. The apparent symptoms of brucellosis were also included. Then, blood specimens were taken for *Brucella melitensis* testing.

Serological test

A 5 cc specimen of CaEDTA blood was taken from the antecubital area of the forearm of every volunteer. It was subsequently transferred to the laboratory at HRH MSMC and analyzed. Fresh serum was obtained by centrifugation of the clotted blood. The serum samples were screened for antibodies to *Brucella melitensis* which is the same species of the three cases as evidenced by agglutination tests[6]. The test was performed by use of a pipette, dispensing 0.08 ml, 0.04 ml, 0.02 ml, 0.01 ml and 0.005 ml of undiluted serum onto a row of 3 cm diameter circles. Then, the reagent bottle was shaken and one drop of the undiluted febrile *Brucella melitensis* antigen suspension was added to each serum aliquoat. The specimen was then mixed well using a stirrer and rotating the slide. After one minute, the agglutination in any circle was tested for indications of a seropositive result. The positive titers in 0.08 ml, 0.04 ml, 0.02 ml, 0.01 ml and 0.005 ml of serum aliquots refer to positive titers of 1:20, 1:40 and 1:80, 1:160 and 1:320 dilutions, respectively.

Statistical analysis

Demographic data and possible risk factors were collected and classified by categorical data using frequency and percentage. Univariable analysis was utilized to determine the association between seropositivity and each possible risk factor. Multicollinearity was checked prior to multivariate analysis. Then multiple logistic regression analyses were utilized to adjust any possible confounding factors. Enter procedures were used in modeling. Finally, the appropriateness was checked by Wald and Hosmer-Lemeshow's goodness-of-fit test. Odds ratio with 95% confidence interval (CI) were presented to ascertain statistical significance as to the association of seropositivity to *Brucella melitensis*[7].

Results

All 86 volunteers participated in the present study; their mean ages were 51.90 $\pm$ 16.28 years old, ranging from 18-85 years of age. 58.14% were females and 41.86% were males. As for their living areas, 45.35% resided in goats and cattle feeding areas, 31.40% resided in the same area of the first index case and 23.25% lived in no livestock feeding areas, respectively. Most of their occupations were as business owners or housewives (51.16%). Of all the participants, 39 persons had seropositive titers to *Brucella melitensis* yielding a prevalence rate of 45.35% (95%CI; 34.61-56.08%). Of these; 35.90% had titers at 1:20 dilution, 56.41% had titers at 1:40 dilution, 5.13% had titers at 1:80 dilution and 2.56% had titers at 1:320 dilution. All of the seropositive subjects did not show clinical symptoms of brucellosis. The factors associated with seropositive conversion before and after adjusted confounders are as shown in Table 1.

Discussion

Human brucellosis is a rare disease in Thailand. However, it could sporadically occur in areas where it has never been reported before. If proper control systems are not established, it could be a major public health concern. The objective of this serological survey was to evaluate the seroprevalence and the factors associated with seropositive titers of *Brucella melitensis* among residents who live in a community after an outbreak of this disease in order to control and prevent future epidemics. All of these potential risk factors *i.e.* foodborne transmission, contaminated environment and occupational exposure through direct or indirect contact were investigated in the present study. The results show the high seropositive titers in the study group. However, this study did not find any subjects who had symptoms relating to brucellosis. The high seropositive titers to *Brucella melitensis* may be from a selection of high risk villages. Most residents in the study areas are Muslims whose habits, *i.e.*, feeding goats, consumption of raw goat milk and meat

Table 1. Crude and adjusted odds ratio (ORs) with 95% confidence interval (CI) of factor associated with seropositive antibodies to *Brucella melitensis*

Factors	Seropositve/ Seronegative (n)	Crude odds ratio (95%CI)	Adjusted odds ratio (95%CI)
Age group			
- < 45 years old	12/22	1.00	1.00
- 45-60 years old	16/10	2.93 (1.02-8.45)	3.02 (0.81-11.37)
- > 60 years old	11/15	1.34 (0.47-3.84)	1.53 (0.35-6.71)
Gender			
- Male	15/21	1.00	1.00
- Female	24/26	1.29 (0.55-3.07)	2.24 (0.66-7.57)
Occupation			
- Business owner /housewife	17/27	1.00	1.00
- Farmer	20/15	2.12 (0.86-5.23)	1.72 (0.49-6.01)
- Animal feeder	2/5	0.64 (0.11-3.65)	0.11 (0.01-1.65)
Area of study			
- Being in a village that no livestock feeding	12/8	1.00	1.00
- Being in a village that had goats and cattle feeding	20/19	0.70 (0.24-2.09)	0.80 (0.17-3.74)
- Being in a village that the same area of the first index case	7/20	0.23 (0.07-0.81)	0.48 (0.08-2.77)
Contact with goats			
- No	25/36	1.00	1.00
- Yes	14/11	1.83 (0.72-4.69)	2.64 (0.99-6.97)
Contact with cattle			
- No	24/38	1.00	1.00
- Yes	15/9	2.64 (0.99-6.97)	1.20 (0.26-5.54)
Contact with labored or aborted goats			
- No	35/46	1.00	1.002
- Yes	4/1	5.26 (0.56-49.13)	27.16 (1.02-721.53)
Slaughtering or butchering goats			
- No	22/28	1.00	1.00
- Yes	17/19	1.14 (0.48-2.69)	1.54 (0.42-5.62)
Consumption of raw goat milk or meat			
- No	24/37	1.00	1.00
- Yes	15/10	2.31 (0.89-5.99)	6.27 (1.25-31.36)
Use goat feces as fertilizer			
- No	27/34	1.00	1.00
- Yes	12/13	1.29 (0.49-3.39)	1.56 (0.25-9.77)
Use cattle feces as fertilizer			
- No	32/41	1.00	1.00
- Yes	7/6	1.50 (0.46-4.89)	7.60 (0.62-91.83)
Use soil from goat feeding area to planting			
- No	25/30	1.00	1.00
- Yes	14/17	0.99 (0.41-2.39)	0.63 (0.15-2.67)
Usually walk to goat or cattle farm			
- No	17/21	1.00	1.00
- Yes	22/26	1.05 (0.44-2.46)	1.63 (0.44-6.00)
Knowledge of brucellosis			
- No	31/38	1.00	1.00
- Yes	8/9	1.09 (0.38-3.16)	1.67 (0.36-7.67)
Personal hygiene			
- Good	32/40	1.00	1.00
- Fair	7/7	1.25 (0.40-3.93)	1.15 (0.27-4.98)

could lead to brucellosis infections. However, the sensitivity to false positive reactions of a serum agglutination test should not be excluded. It may be confounded by exposure to cross reactions of common microorganisms, including *Salmonella* 0:30, *Escherichia coli* 0:157 and *Yersinia enterocolitica* 0:9[8]. All seropositivity should be confirmed with more specific tests. This method is, nevertheless, commonly used for the detection of *Brucella spp.* antibodies in field study because it is inexpensive, simple and can be used as a rapid screening tool in a community setting[9].

As for risk factors, the results revealed that contact with labored or aborted goats increased the risk of generating seropositive titers. It may be that *Brucella spp.* can spread the bacilli by mucosa or abraded skin among victims. Consumption of raw goat products can infect the human body by penetration of the gastric mucosa through ingestion. After entering the human body, *Brucella spp* is up taken by local tissue lymphocytes, transferred to regional lymph nodes into the circulation, and subsequently disseminated throughout the body. The disease usually manifests as an acute febrile illness which may persist and progress to a chronically incapacitating disease with complications. In the present study, there was some limitation during the study in the form of a recall bias from an interview of risk factors potentially causing an information bias. However, the factors associated with brucellosis infection are consistent with other areas experiencing outbreaks in Thailand and the endemic countries[4,10-13]. If these risk behaviors are not prevented, it could cause epidemics of this disease in our country in the future. On the other hand, cleansing farms, understanding the brucellosis pathogen and personal hygiene were not significant preventative factors in the present study. Nonetheless, the results show that few residents have had the knowledge about brucellosis disease and some residents had high risk behaviors that could aid in infection of this disease. Therefore, hygienic practices are still a key factor in reducing the likelihood of a herd being infected and preventing this problem. For actions taken after study, the authors provided knowledge about brucellosis for residents and local public health officers. The appropriate prevention measures, ranging from health education to proper sanitation practices, the use of personal protection especially during contact with contaminated animal parts and their by products as well as education on consuming heated goat milk or cooked meat were implemented. The authors also raised awareness about the brucellosis disease to physicians in our hospital.

Brucellosis could cause enormous economic losses due to the loss of infected animals as well as considerable human morbidity and mortality. Human brucellosis causes nonspecific symptoms and clinical manifestations which make it difficult to diagnose and not clinically detected by physicians. In high risk or outbreak areas, physicians and public health personnel should be aware of the possibility of infection from brucellosis. Physicians should have a high degree of suspicion when evaluating patients presenting with prolonged fevers and having the risk factors of this disease. A surveillance system, including report to the national communicable disease surveillance system is an important step. After having found the index case in a hospital, public health personnel should have community investigation teams to identify the cause, the risk factors and work to identify other silent cases[14]. Not doing these activities might prevent effective disease monitoring, disease prevention and hinder control efforts for the spread of brucellosis.

In conclusion, this community based study found a high seropositive prevalence of brucellosis which had never been reported in this area before. The factors associated with seropositivity are the contact with labored or aborted goats and consumption of raw goat products. These factors are similar to the other outbreak areas in Thailand and the endemic countries. Relevant local authorities should be encouraged in promoting health education to aid in prevention and control of this problem.

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Potential conflicts of interest

None.

References

1. Bruce D. Note on the discovery of a microorganism in Malta fever. Practioner 1887; 39: 161-70.
2. Pappas G, Papadimitriou P, Akritidis N, Christou L, Tsianos EV. The new global map of human brucellosis. Lancet Infect Dis 2006; 6: 91-9.
3. Manosuthi W, Thummakul T, Vibhagool A, Vorachit M, Malathum K. Case report: Brucellosis: a re-emerging disease in Thailand. Southeast Asian J Trop Med Public Health 2004; 35: 109-12.

4. Paitoonpong L, Ekgatat M, Nunthapisud P, Tantawichien T, Suankratay C. Brucellosis: the first case of King Chulalongkorn Memorial Hospital and review of the literature. J Med Assoc Thai 2006; 89: 1313-7.
5. Buppanharun W, Santiwatanakul S, Chansiri K, Varavithya V, Wongamorntham S, Tantisiriwat W. Epidemiology of brucellosis at HRH Princess Sirindhorn Medical Center, Srinakharinwirot University. Proceedings of 10th Western Pacific Congress on Chemotherapy and Infectious Diseases. ; 2006 December 3-6; Fukuoka. Fukuoka: The Japanese Society of Chemotherapy, 2006.
6. Cobel MJ. Brucellosis in humans and animals. Geneva: World Health Organization; 2006.
7. Chan YH. Biostatistics 202: logistic regression analysis. Singapore Med J 2004; 45: 149-53.
8. Moreno E, Cloeckaert A, Moriyon I. Brucella evolution and taxonomy. Vet Microbiol 2002; 90: 209-27.
9. Sharma R, Chisnall C, Cooke RP. Evaluation of in-house and commercial immunoassays for the sero-diagnosis of brucellosis in a non-endemic low prevalence population. J Infect 2008; 56: 108-13.
10. Reid AJ. Brucellosis—a persistent occupational hazard in Ireland. Int J Occup Environ Health 2005; 11: 302-4.
11. Kozukeev TB, Ajeilat S, Maes E, Favorov M. Risk factors for brucellosis—Leylek and Kadamjay districts, Batken Oblast, Kyrgyzstan, January-November, 2003. MMWR Morb Mortal Wkly Rep 2006; 55 (Suppl 1): 31-4.
12. Abo-Shehada MN, Odeh JS, Abu-Essud M, Abuharfeil N. Seroprevalence of brucellosis among high risk people in northern Jordan. Int J Epidemiol 1996; 25: 450-4.
13. Mishal J, Ben Israel N, Levin Y, Sherf S, Jafari J, Embon E, et al. Brucellosis outbreak: analysis of risk factors and serologic screening. Int J Mol Med 1999; 4: 655-8.
14. Almuneef MA, Memish ZA, Balkhy HH, Alotaibi B, Algoda S, Abbas M, et al. Importance of screening household members of acute brucellosis cases in endemic areas. Epidemiol Infect 2004; 132: 533-40.

ปัจจัยที่สัมพันธ์กับผลเลือดที่มีแอนติบอดีต่อเชื้อ *Brucella melitensis* ในอำเภอองครักษ์ จังหวัดนครนายก ประเทศไทย

ฉัตรชัย เอกปัญญาสกุล, สมชาย สันติวัฒนกุล, วรพจน์ ตันติศิริวัฒน์, วันชัย บุพพันเหรัญ

ภูมิหลัง: *โรคบลูเซลโลซิสหรือโรคแท้งแพะในมนุษย์เป็นโรคอุบัติซ้ำในประเทศไทย ในปี พ.ศ. 2549 โรงพยาบาลศูนย์การแพทย์ สมเด็จพระเทพรัตนราชสุดาฯ จังหวัดนครนายก ได้วินิจฉัยผู้ป่วยโรคนี้จำนวน 3 ราย ซึ่งโรคนี้ไม่เคยมีการรายงานในพื้นที่นี้มาก่อน*

วัตถุประสงค์: *เพื่อศึกษาระบาดวิทยาโดยการประเมินความชุกและปัจจัยที่สัมพันธ์กับผลเลือดที่มีแอนติบอดีต่อเชื้อ Brucella melitensis ในชาวบ้านที่อาศัยในตำบลเดียวกับผู้ป่วยรายแรก*

วัสดุและวิธีการ: *ใน พ.ศ. 2550 ได้ดำเนินการศึกษาในตำบลชุมพล อำเภอองครักษ์ จังหวัดนครนายก ประเทศไทย ซึ่งเป็นพื้นที่ที่เคยมีการระบาดของโรคในปีที่ผ่านมา โดยเก็บข้อมูลในชาวบ้านจำนวน 86 ราย ที่คัดเลือกมาจากสามหมู่บ้านในพื้นที่ที่ศึกษา เก็บเลือดและทดสอบการมีแอนติบอดีต่อเชื้อ Brucella melitensis ด้วยเทคนิค serum agglutination ร่วมกับการซักประวัติปัจจัยเสี่ยงโดยใช้การสัมภาษณ์จากแบบสอบถามแบบมีโครงสร้าง วิเคราะห์ข้อมูลทางสถิติด้วยความถดถอยแบบโลจิสติก*

ผลการศึกษา: *จากตัวอย่างทั้งหมดที่ศึกษาพบอัตราความชุกของผลเลือดที่มีแอนติบอดีต่อเชื้อ Brucella melitensis ร้อยละ 45.35 (ค่าความเชื่อมั่นที่ร้อยละ 95 = ร้อยละ 34.61-56.08) เมื่อปรับปัจจัยรบกวนต่างๆ พบว่าปัจจัยที่สัมพันธ์กับการมีผลเลือดเป็นบวกได้แก่ การสัมผัสแพะที่เพิ่งคลอดหรือแท้งมีค่าความเสี่ยงเท่ากับ 27.16 เท่า (ค่าความเชื่อมั่นที่ร้อยละ 95 = 1.02-721.53 เท่า) และการบริโภคผลิตภัณฑ์ดิบๆ ของแพะ มีค่าความเสี่ยงเท่ากับ 6.27 (ค่าความเชื่อมั่นที่ร้อยละ 95 = 1.25-31.36 เท่า)*

สรุป: *การศึกษานี้พบว่าความชุกต่อการมีผลเลือดเป็นบวกต่อเชื้อ Brucella melitensis สูงหลังมีการระบาดของโรคในปี พ.ศ. 2549 โดยพบว่าปัจจัยที่สัมพันธ์กับการติดเชื้อดังกล่าวคือ การสัมผัสโดยตรงกับสัตว์ที่เป็นโรค ซึ่งเหมือนกับพื้นที่ที่มีการระบาดในประเทศไทยและประเทศที่มีการระบาดเป็นประจำ ดังนั้นบุคลากรทางสุขภาพที่เกี่ยวข้องในพื้นที่นอกจากจะดำเนินการให้สุขศึกษาในชุมชนแล้ว ควรเฝ้าระวังโรคอย่างต่อเนื่องเพื่อช่วยการควบคุมและป้องกันการระบาด*