Short Communication

Serological Detection of *Brucella* Infection in Small Ruminant in Nhong-Mamong District, Chainat Province, Thailand

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Abstract

Brucellosis stands as a significant zoonotic disease of concern. In Thailand, the first case of human brucellosis emerged in 2003, attributed to goat milk consumption. Subsequently, in 2007, there was a resurgence of brucellosis, which heightened awareness of its implications for public health. Our study was undertaken as part of the 17th Veterinary Volunteer Project in the Nhong-mamong district, Chainat province from June 21 to June 30, 2023. The aim was to assess the seropositivity of brucellosis in goats and sheep in this area. A total of 649 serum samples were collected from 15 farms and examined using the Rose Bengal test. Our findings revealed that the seropositivity at the farm and individual levels was 13.33% (2/15) and 0.77% (5/649), respectively. Consequently, it became evident that the study area remained at risk for brucellosis infection, posing a threat to both humans and animals, particularly concerning *Brucella melitensis* in goats and sheep, which could lead to severe illness in humans. Consequently, an early warning surveillance system is essential to protect both animals and public health by providing timely knowledge and control measures.

Keywords: Brucellosis, Seropositive, Small ruminant, Chainat province

ผลบวกทางซีรัมวิทยาต่อโรคติดเชื้อบรูเซลลาในสัตว์เคี้ยวเอื้องขนาดเล็กของอำเภอหนองมะโมง จังหวัดชัยนาท ประเทศไทย

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บทคัดย่อ

โรคบรูเซลโลซีสเป็นโรคติดต่อระหว่างสัตว์และคนที่สำคัญ ประเทศไทยมีการรายงานผู้ป่วยโรคบรูเซลโลซีสรายแรกเมื่อปี พ.ศ. 2546 จากการสอบสวนโรคผู้ป่วยได้รับเชื้อจากการบริโภคนมแพะ ต่อมาในปี พ.ศ. 2550 มีการรายงานผู้ป่วยติดเชื้อโรคบรูเซลโลซิสอีกครั้ง ทำให้หน่วยงานสาธารณสุขเกิดความตระหนักถึงผลกระทบของโรคนี้ต่อสุขภาพของประชาชนมากขึ้น การศึกษาครั้งนี้ได้จัดทำขึ้นภายใต้โครงการสัตวแพทย์อาสา คณะสัตวแพทยศาสตร์ ครั้งที่ 17 ระหว่างวันที่ 21-30 มิถุนายน พ.ศ. 2566 ณ อ. หนองมะโมง จ. ชัยนาท เพื่อศึกษาผลบวกทางซีรัมวิทยาของโรคบรูเซลโลซีสในแพะและแกะ ตัวอย่างซีรัมแพะและแกะทั้งหมด 649 ตัวอย่าง จากฟาร์มแพะและแกะใน อ. หนองมะโมง จำนวน 15 ฟาร์ม ตัวอย่างซีรัมตรวจด้วยวิธี Rose Bengal test ผลปรากฏว่า ผลบวกทางซีรัมต่อโรคบรูเซลโลซีสรายฟาร์มและรายตัวมีค่าเท่ากับ ร้อยละ 13.33 (2/15) และ ร้อยละ 0.77 (5/649) ตามลำดับ แสดงให้เห็นว่าพื้นที่ที่ศึกษายังมีโอกาสเกิดโรคบรูเซลโลซีส ซึ่งเป็นอันตรายต่อทั้งมนุษย์และสัตว์โดยเฉพาะเชื้อ ***Brucella melitensis*** ในแพะและแกะ ซึ่งอาจนำไปสู่การเจ็บป่วยในมนุษย์ที่รุนแรงได้ ดังนั้นการมีระบบเฝ้าระวัง มาตรการการป้องกัน และการให้ความรู้แก่เกษตรกรจึงมีความจำเป็นในการป้องกันโรคทั้งในสัตว์และคน

คำสำคัญ : โรคบรูเซลโลซีส ผลบวกทางซีรัมวิทยา สัตว์เคี้ยวเอื้องขนาดเล็ก ชัยนาท

Introduction

Brucellosis is a highly contagious zoonotic disease with significant public health and economic implications (Wongsawang et al., 2014; Hull and Schumaker 2018). The World Organization for Animal Health (formerly known as OIE) recognizes brucellosis as one of the important zoonotic diseases responsible for human and animal illnesses, as well as detrimental effects on livestock production and trade. This underscores the need for robust public health monitoring (OIE 2019).

In Thailand, the Department of Livestock Development (DLD) has launched the "Brucellosis Free" campaign, which covers various livestock categories, including dairy cattle, goats, and sheep (Colombe et al., 2018). Additionally, they have established regulations for certifying farms as brucellosis-free, necessitating that goat and sheep farms be free from brucellosis.

Brucellosis is caused by small, gram-negative, non-motile, non-spore-forming coccobacilli bacteria in the genus *Brucella*, which are resilient in various environmental conditions, including cold temperatures, yet susceptible to destruction. In cattle, brucellosis is primarily caused by *B. abortus*. *B. melitensis*, and *B. ovis* are the main responsible pathogens in goats and sheep, respectively. In addition, *B. suis* is mostly found in swine, dogs can be infected with *B. canis* (Corbel 2006) and *B. neotomae* is the *Brucella* spp. frequently found in rodents (Villalobos-Vindas et al., 2017). *Brucella* infection can occur through ingestion via contaminated food and water, contact with vaginal discharge, or milk. Furthermore, mating with an infected animal, particularly a male goat, can swiftly spread the infection among female animals within the herd (Godfroid et al., 2011). Once inside the body, *Brucella* pathogens disseminate to lymph nodes and vital target organs, including the uterus and placenta, resulting in diverse manifestations, including abortion in females, retained placenta, arthritis, orchitis in males, and epididymitis (Ekgatat 2009; Kladkempetch et al., 2018).

Brucella melitensis is the most common *Brucella* pathogen that causes human brucellosis, followed by *B. abortus*, *B. suis*, and *B. canis*, respectively (Wallach et al., 1998). The incubation period of human brucellosis is varied, with the most frequent clinical signs being intermittent or irregular fever, headaches, weakness, joint pain, depression, and weight loss (Corbel 2006). *Brucella* infection in humans is mainly linked to occupational exposure, such as laboratory work or jobs involving direct contact with animals, like veterinarians and farmers (Ekgatat 2009). Studies on risk factors have revealed that direct contact with calving discharges, contact through wounds, exposure via the conjunctival sac of the eyes, inhalation of infectious aerosols, and even the consumption of dairy products, such as milk and cheese, pose a high risk of infection (Burns et al., 2023). Lewis et al. documented a case of a 51-year-old UK resident who was infected with *B. melitensis* after returning from Thailand in 2015 (Lewis et al., 2016), drawing attention to the risks associated with brucellosis and prompting efforts to educate the public about relevant knowledge and risk factors, both in the medical and veterinary fields.

In Thailand, brucellosis had not been reported since 1970 until 2003, when the Ministry of Public Health (MOPH) identified and documented the re-emergence of human brucellosis. The affected patient worked at a goat farm in Ratchaburi province and had a history of consuming goat milk and raw goat meat (Laosiritaworn et al., 2007). In 2007, three cases were reported in Chainat province and one in Uttaradit

province. All patients had a history of animal contact and consumption of animal products, particularly from small ruminants. Furthermore, in 2010, 11 brucellosis cases were reported across Nakhon Pathom, Ratchaburi, Rayong, Chainat, and Phetchaburi provinces. Nine were diagnosed via blood culture, while three were examined using the Rose Bengal Test (Annual epidemiological surveillance report 2010). Additionally, direct contact with goat secretions or blood, especially without protective equipment, or consuming goat meat, can transmit brucellosis to humans (Wongphruksasoong et al., 2012; Kanitpun et al., 2022). Consistent with Kanitpun et al. (2022), this study found that goats and sheep had the highest percentage of seropositive results for brucellosis, indicating that they are susceptible to the disease. However, the DLD's efforts in promoting goats and sheep farming, with a focus on brucellosis prevention and control, have had a positive impact, improving the situation of brucellosis in Thailand (Kanitpun et al., 2022).

In Chainat province, goat and sheep farming promotion was aligned with the DLD's policies. A goat farmers' group was formed and provided farmers with five female and one male goat each. Consequently, the population of goats and sheep in Chainat province experienced a rapid increase, accompanied by the detection of brucellosis in meat goats in Chainat (Suddee et al., 2011). However, the province in the central and western region of Thailand with the highest number of goat and sheep tests from 2013 to 2015 was Kanchanaburi (Peck et al., 2018).

This study thus aimed to examine the serological status of goats and sheep regarding *Brucella* infection in the Saphan Hin subdistrict of Nhong-mamong district, Chainat province, and raise awareness on prevention and control of the disease among relevant authorities and farmers.

Materials and methods

Study location

We performed a cross-sectional study to explore the serological status of *Brucella* infection in goats and sheep in Saphan Hin subdistricts, Nhong-mamong district, Chainat province, Thailand with a previous history of outbreak event (Suddee et al., 2011). The study was conducted on 21st-30th June 2023 under the 17th Veterinary Volunteer Project by the Faculty of Veterinary Science, Mahidol University, with the collaboration of the Nhong-mamong District Livestock Office, Chainat province.

Sample collection and laboratory diagnosis

We collected serum samples from goats and sheep older than six months on 14 goat farms and one sheep farm (Table 1) in Saphan Hin subdistrict, Nhong-mamong district, Chainat province (Figure 1). We collected 5 ml blood samples from the jugular vein of each animal. The samples were then examined for antibodies against *Brucella* spp. infection using the Rose Bengal Test (RBT). This test is based on the principle of agglutination between the antigen and antibody under acidic conditions, with a sensitivity and specificity of 99% and 97%, respectively (Thuamsuwan and Yoopracham 2021). The ratio of serum to the Rose Bengal solution is 75 µl:25 µl. The serum and solution were mixed thoroughly on a clean glass plate, which was gently shaken before the results were read after four minutes. Test results were recorded as either negative (no agglutination) or positive (+1 to +4, depending on the degree of agglutination).

Descriptive statistics and percent positivity at a 95% confidence interval (CI) were calculated using an online platform available at https://www.statskingdom.com/proportion-confidence-interval-calculator.html.

Table 1. Number of tested farms and animals for brucellosis in the Saphan Hin subdistrict, Chainat province.

Level	Results	Total tested samples	Moo1	Moo4	Moo6	Moo7	Moo9
Farm	Overall	15	2	7	3	2	1
	Goats	14	2	7	2	2	1
	Sheep	1	0	0	1	0	0
Individual	Overall	649	34	324	165	47	79
	Goats	598	34	324	114	47	79
	Sheep	51	0	0	51	0	0

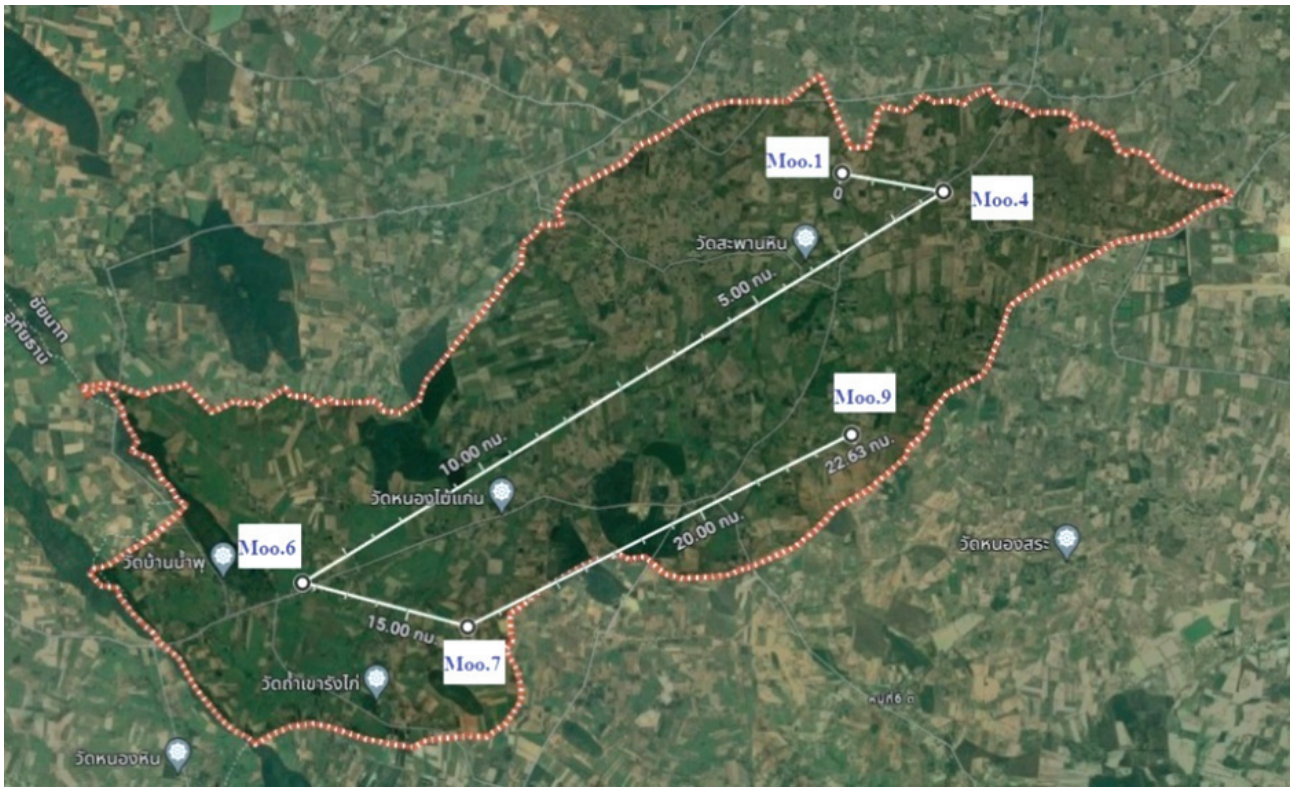

Figure 1. Locations of goat and sheep farms included in the study in Saphan Hin subdistrict, Nhong-mamong district, Chainat province, Thailand. (Google maps 2023).

Results

In total, 649 samples were collected from 14 goat farms and one sheep farm, in which two farms were found to have seropositivity (farm-level seropositivity: 2/15; 13.33%; (95% CI = 3.7-38.0). At the individual level, five samples were found seropositive (individual-level seropositivity: 5/649; 0.77%; (95% CI = 0.33-1.80). Moo (Village) 6 found the highest seropositive level, whereas no positive samples were found in Moo 1, Moo 7, and Moo 9 (Table 2). Concerning the species of the animals, sheep showed a higher seropositive level on both farm and individual levels (Table 3).

Table 2. Seropositive results of *Brucella* infection in small ruminants in each village (Moo) within Nhong-mamong district, Chainat province.

Level	Results	Nhong-mamong district, Chainat province					
		2023	Moo1	Moo4	Moo6	Moo7	Moo9
Farm	Overall	15	2	7	3	2	1
	Positive	2	0	1	1	0	0
	Seropositive	13.33%	0	14.29%	33.33%	0	0
	95%Cl	3.7-38	0	2.6-51	6.1-79	0	0
Individual	Overall	649	34	324	165	47	79
	Positive	5	0	1	4	0	0
	Seropositive	0.77%	0	0.30%	2.42%	0	0
	95%Cl	0.33-1.8	0	0.05-1.7	0.95-6.1	0	0

Table 3. Seropositive results of *Brucella* infection in each animal species in Nhong-mamong district, Chainat province.

Level	Results	Total tested samples	Positive	Seropositive	95%Cl
Farm	Overall	15	2	13.33%	3.7-38
	Goats	14	1	7.14%	1.3-31
	Sheep	1	1	100%	21-100
Individual	Overall	649	5	0.77%	0.33-1.8
	Goats	598	1	0.16%	0.03-0.94
	Sheep	51	4	7.84%	3.1-18

Discussion

Our study on brucellosis seropositivity in Saphan Hin subdistrict, an area densely populated with goat farms within Nhong-mamong district of Chainat province, revealed that most of these farms are small-scale and operate on a free-range basis, with an average of 44 goats per farm. There is a mix of both old and new farmers raising goats and sheep. Specifically, the newly raised farms are the first survey of brucellosis.

The seropositive tests revealed at least one farm and one animal within a goat herd tested positive in Nhong-mamong district, Chainat, Thailand. Additionally, a sheep herd had the highest seropositive result. It was 100% (95%Cl:21-100) at the herd level and 7.84% (95%Cl:3.1-18) at individual level. It might cause only one sheep farm in Nhong-mamong district, Chainat and brucellosis screening and quarantine information remain unclear. It is essential to conduct further testing and investigation to confirm the presence of brucellosis and assess the extent of the infection. Proper diagnosis and control measures are crucial to prevent the spread of the disease within the herd and to other animals or potentially to humans.

Our study also noted a higher trend of seropositivity in Nhong-mamong district compared to the findings in a previous study conducted in 2011 by Suddee et al. According to the previous study, the percentage of positive results at the herd level was 11.11% with 0.14% at the individual level (Suddee et al., 2011). This increase in seropositivity is a concerning trend as it may indicate a higher prevalence of the disease in Nhong-mamong district. Brucellosis outbreaks in goats and sheep across the entire country are primarily attributed to insufficient preventive measures and the introduction of new animals into herds from farms with unknown brucellosis status can be a risk factor for the spread of the disease (Kanitpun et al., 2022). As the movement of livestock and human workforces increases, there is a higher risk of brucellosis spreading to new areas (Colombe et al., 2018). These factors significantly contribute to disease incidence among animals. Given that *Brucella* pathogen can be directly transmitted from animals to humans through unhygienic practices such as consuming unpasteurized milk or raw meat products (Godfroid et al., 2011). Direct contact with infected animals or their calving secretions without the use of protective gloves or gear, and farmers' limited knowledge about zoonotic diseases, a public educational campaign should be urgently initiated in the area to prevent the occurrence of possible human infection (Annual epidemiological surveillance report 2010). Moreover, it is crucial to enhance awareness of brucellosis and conduct annual surveillance in both livestock and humans to successfully eliminate brucellosis from Chainat province. Achieving this goal will require ongoing collaboration between DLD, the medical community, veterinary schools, and private farmers to sustain brucellosis surveillance and prevention programs. Vaccination and treatment are possible for brucellosis in

animals, but the use of live vaccines presents significant safety concerns, such as the potential to induce abortion, pathogenicity for humans, interference with serodiagnosis, and antibiotic resistance. (Elbehiry et al., 2023) As a result, the preferred approach for brucellosis control and prevention involves regular monitoring and culling of infected animals. This strategy helps reduce the risk of disease transmission while safeguarding both animal and human health (Ullah et al., 2020).

We acknowledge some limitations faced in this study. Firstly, the study was conducted as part of the Veterinary Volunteer Project, which may have led to an inadequate sample size calculation. For future studies, we highly recommend employing more robust sampling techniques and accurately calculating the sample size to ensure the study population is proper representation. Additionally, we suggest considering a longitudinal study to continuously monitor the disease incidence trend in the study area, enabling early detection of any unexpectedly high incidence rates.

In conclusion, *Brucella* infection was serologically detected in goats and sheep in a certain area in Chainat province. Hence, it is imperative to establish comprehensive control and prevention measures, along with an annual surveillance campaign, in alignment with the "Brucellosis Free" initiative by the DLD to work towards eradicating brucellosis from these herds.

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