

Risk factors associated with seroprevalence of paratuberculosis in mature goats in Northeastern Thailand

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Abstract

The aim of this study was to investigate risk factors for the seroprevalence of paratuberculosis in mature goats in Northeastern Thailand. The study area included Buriram, Chaiyaphum, Kalasin, Khon Kaen, Mahasarakham, Nakhonratchasima, Roi et, Sakon Nakhon, Srisaket, and Udonthani. The seroprevalence of paratuberculosis determined in 37 herds, a total of 671 serum samples, was 12.82%. The risk factors related to paratuberculosis were analyzed at individual level using mixed model logistic regression. At the individual level, 86 of 671 goats tested positive based on an ELISA assay. Risk factors associated with the seroprevalence of MAP antibodies included age, divided into six groups, sex, breed, and body condition score (BCS). In addition, when using goats younger than 1 year old as a baseline, older goats were at higher risk than younger goats: 3 years old [$(p < 0.05)$; OR = 5.48; 95% CI = 1.17-25.64], 4 years old [$(p < 0.01)$; OR = 8.55; 95% CI = 1.89-38.10], and over 5 years old [$(p < 0.01)$; OR = 18.05; 95% CI = 3.20-102.01]. Both male and female goats and goats of different breeds were at similar risk of paratuberculosis based on the insignificant difference results ($p > 0.05$). The results reveal that paratuberculosis is prevalent in the goat herds in Northeastern Thailand. Age is identified as a factor that should be monitored in terms of disease outbreak and economic losses due to disease.

Keywords: paratuberculosis, ELISA, goats, risk factors, Northeastern Thailand

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Introduction

Paratuberculosis, also known as Johne's disease (JD), is caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). It can induce chronic granulomatous enteritis in ruminants. According to a report by the World Organisation for Animal Health (OIE), paratuberculosis is present worldwide in domestic and wild animals, including in Europe, America, Africa, Asia, and Australia (Michel and Bastianello, 2000; Lee and Jung, 2009; Nielsen and Toft, 2009; Lombard, 2011; OIE-World Organisation for Animal Health, 2016). Paratuberculosis is among the diseases listed as affecting multiple species by OIE in their 2017 report. The reported prevalence of MAP infection in small ruminants in Latin America and the Caribbean is 4.3% (95% CI, 1.9-6.8) at the animal level and 3.7% (95% CI, 0.1-7.4) at the goat herd level (Fernández-Silva et al., 2014). In Germany, 65% of small ruminant flocks tested seropositive for paratuberculosis and 21% of individual goats were serologically positive against MAP in 2009 (Angelika et al., 2012).

MAP infection may result in increased morbidity and mortality, reduced reproductive performance, and economic losses (Lawrence, 1996; Polychronis et al., 2006). In addition, paratuberculosis is significantly associated with Crohn's disease (CD) in humans (Samin et al., 2017).

The mode of transmission of MAP infection is primarily the fecal-oral route by ingestion of food and water contaminated with feces of MAP-infected animals; it may also be transmitted by contaminated colostrum and milk from a mother to newborn animals (Peter and Richard, 2010). Due to the slow progression of MAP, infected animals may not show signs of infection for as long as two years. MAP causes chronic intestinal inflammation, presenting as diarrhea and progressive weight loss in spite of a good appetite and normal body temperature (Mary and David, 2009). Chronic enteritis can also lead to the functional loss of proteins and the inability to absorb proteins, which leads to muscle wasting and low production. These may cause bottle jaw due to hypoproteinemia (Mary and David, 2009). Other commonly found symptoms in MAP-infected goats include signs of progressive weight loss, cachexia, and weakness in the last stage of illness (Elizabeth and Michael, 2001).

Based on the previous study, the seroprevalence of paratuberculosis infection was 12.82% in small goat herds in Northeastern Thailand (Sarinya et al., 2017). The goal of this study was to investigate individual level risk factors associated with the seroprevalence of paratuberculosis in mature goats in Northeastern Thailand.

Materials and Methods

Study design and study area: A cross-sectional study was conducted to determine the seroprevalence of paratuberculosis in goat herds from April to August of 2016. Provinces in which there were over 25 goat herds were selected for the study, including Buriram, Chaiyaphum, Kalasin, Khon Kaen, Mahasarakham, Nakhonratchasima, Roi et, Sakon Nakhon, Srisaket, and Udonthani. The study procedures were approved

by the Ethics Committee of the authors' institution, and care was taken to minimize the number of animals used (record no. ACUC KKU 2/2559, reference no. 0514.1.75/4, approval date 21 January 2016).

Sampling: A total of 671 serum samples were randomly selected from 37 small goat herds in the studied provinces. At herd level, herd structure and herd and health management were not significantly different between seropositive herds and seronegative herds. Among these herds, the owners were not aware of paratuberculosis in goats (Table 1). Blood samples were taken from mature goats (over 6 months of age and from both sexes) in each herd using red-top Vacutainer® blood collection tubes (BP-Plymouth, PL6 7BP, UK). All samples were transported on ice to the laboratory at the Faculty of Veterinary Medicine, Khon Kaen University, within four hours of collection. Then, all samples were centrifuged at 2,500 rpm for 10 minutes to collect the serum and were then stored at -20°C until analysis.

Analysis of serum samples: Antibodies against MAP in the serum were detected via CATTLETYPE® MAP Ab ELISA (QIAGEN Leipzig GmbH, Deutscher Platz 5b, D-04103, Leipzig, Germany). The procedure was carried out according to the instructions of the manufacturer of the ELISA kits. Optical densities (OD) of the samples and controls were measured at 450 nm using a spectrophotometer (Bio-Rad (iMark™), Microplate Absorbance Reader, Bio-Rad Laboratories, Inc., USA). S/P ratio was determined on the basis of the OD following the equation:

$$S/P = \frac{(OD_{\text{sample}} - \text{mean}OD_{\text{negative control}})}{(\text{mean}OD_{\text{positive control}} - \text{mean}OD_{\text{negative control}})}$$

Results were classified according to the manufacturer's instructions as follows: S/P ratio ≥ 0.4 positive, and < 0.4 negative.

Statistical analysis: Data were analyzed using Microsoft Office Excel 2013 and STATA, Version 10.0 (STATA Corp., 2007). Mixed model logistic regression in STATA version 10.0 was used for analysis of factors associated with seroprevalence in individual goats. Farm of origin was used as the random effect in the model. Age in years (AGE) was treated as a categorical variable and was divided into six levels [< 1 yr., ≥ 1 to < 2 yr., ≥ 2 to < 3 yr., ≥ 3 to < 4 yr., ≥ 4 to < 5 yr., and ≥ 5 yr.], while body condition score (BCS), breed (BREED), and gender (SEX) were binary variables. Sex was categorized as female (SEX = 1) and male (SEX = 0). Animals with body condition score less than 2.50 were considered unhealthy and classified into one group (BCS = 1), while those that had body condition score equal to or greater than 2.50 were considered healthy goats and included in the other group (BCS = 0) (Mario et al., 2015). Breed was divided into mixed meat breeds, such as Boer and Kalahari red (BREED = 1), and mixed milk breeds, such as Saanen, and Toggenburg (BREED = 0). The univariate analysis was performed in order to identify variables that were associated with the seroprevalence at a significant level of 0.05 as the random effect in the model.

Table 1 Description of herd structure, herd and health management, and farmers' knowledge of paratuberculosis associated with seropositivity of paratuberculosis infection in small goat herds in Northeastern Thailand

Factor	Category	Total (n)	OR	95% CI	p-value
Herd size	<25	22			
	25-45	4	1.20	0.14-10.12	0.87
	≥45	11	1.44	0.34-6.16	0.62
Herd established (mo)	<12	5			
	12 to 24	11	1.25	0.15-10.70	0.84
	>24	21	1.65	0.23-11.99	0.62
Presence of other livestock	Yes	22	1.14	0.31-4.25	0.84
	No	15			
Use of pasture	Yes	25	4.50	0.97-20.83	0.05
	No	12			
Good Agricultural Practice	Approved	2			
	Unapproved	1	1.00	-	1.00
	No assessment	34	0.00	-	1.00
Presence of slaughter house within 5 km	Yes	31	1.41	0.21-9.62	0.73
	No	6			
Veterinary service	Yes	0	NA	NA	NA
	No	37	NA	NA	NA
Use of disinfectants	Yes	31	0.17	0.02-1.58	0.12
	No	6			
Regular cleaning of goat house	Yes	34			
	No	3	0.52	0.04-6.58	0.61
Introduction of new goats to the herd	Yes	36	0.00	-	1.00
	No	1			
Brucellosis testing before introduction to the herd	Yes	24	1.38	0.36-5.34	0.64
	No	13			
Annual Brucellosis testing	Yes	34			
	No	3	0.11	0.00-1.38	0.09
Paratuberculosis testing	Yes	0	NA	NA	NA
	No	37	NA	NA	NA
Goat grouping by age	Separate	3	0.44	0.04-5.38	0.52
	Together	34			
Separated birth area	Separate	23			
	Together	14	1.75	0.11-2.56	0.43
Separation of kids after birth immediately	Yes	0	NA	NA	NA
	No	37	NA	NA	NA
Colostrum feeding	Mother	37	NA	NA	NA
	Milk replacer	0	NA	NA	NA
Mother rearing system	Yes	37	NA	NA	NA
	No	0	NA	NA	NA
Previous history of Paratuberculosis	Yes	14	0.69	0.18-2.62	0.58
	No	23			
Selling of goat	Breeding stock	20			
	Meat	15	2.25	0.57-8.82	0.25
	Never	2	1.50	0.08-27.61	0.79
Knowledge about paratuberculosis in goat	Some	0	NA	NA	NA
	None	37	NA	NA	NA

Results

Individual and herd seroprevalence: Of the 671 serum samples tested, 86 were found positive for MAP antibodies, with an apparent prevalence of 12.82% and a true prevalence of 13.28%. The intra-herd seroprevalence ranged from 5.00 to 50.00 (95% CI: 8.83-16.69%) in 29 positive herds out of 37 total herds. Figure 1 shows a map of the distribution of paratuberculosis in goat herds in Northeastern Thailand.

Univariate analysis: The statistical results showed that only age was significantly associated with the seroconversion of the disease (Table 2). Animals

remained at the same risk until 2 years old ($p > 0.05$), after which the risk increased every year from 5.5 in year 3 [$(p < 0.05)$, OR = 5.48; 95%CI = 1.17-25.64] to 8.5 in year 4 [$(p < 0.05)$, OR = 8.50; 95%CI = 1.89-38.10] and 18.0 in years 5 and up [$(p < 0.05)$, OR = 18.05; 95%CI = 3.19-102.01] compared to animals younger than 1 year. Male and female animals were at same risk of getting the disease, as evidenced by the lack of significance of this variable [$(p > 0.05)$, OR = 0.77; 95%CI = 0.35-1.74]. BCS was not associated with the seroconversion results [$(p > 0.05)$, OR = 1.16; 95%CI = 0.70-1.91]. All mixed breeds were also at the same risk of seropositivity for paratuberculosis, as the difference did not reach a significant level [$(p > 0.05)$, OR = 0.66; 95%CI = 0.22-1.94].

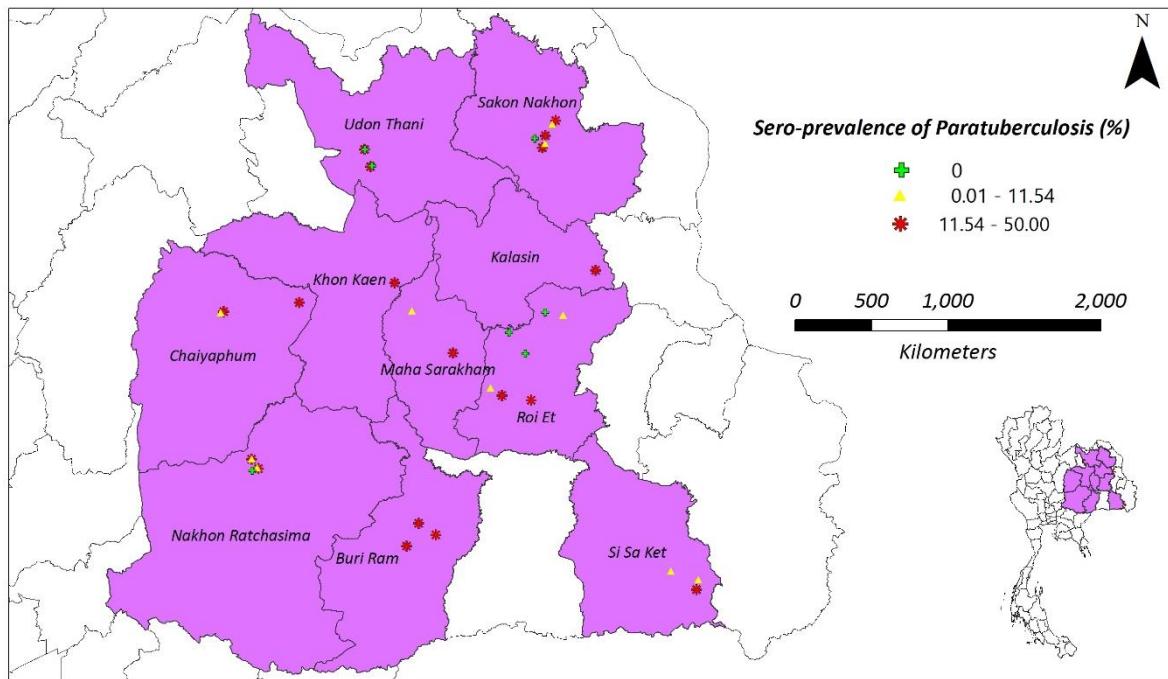


Figure 1 Map showing the distribution of caprine paratuberculosis in herds of small ruminants in Northeastern Thailand (Sarinya et al, 2017)
Green = herd free from paratuberculosis, Yellow = seroprevalence in herd less than or equal to 11.54%, Red = seroprevalence greater than 11.54%

Table 2 Univariate analysis of seropositivity for paratuberculosis infection at individual level

Factor	Category	Number	Positive (%)	OR	95% CI	p-value
Age	<1 yr	66	2 (3.0)			
	≥1 - <2 yr	141	8 (5.7)	1.77	0.34-9.07	0.49
	≥2 - <3 yr	94	4 (4.3)	1.30	0.22-7.72	0.78
	≥3 - <4 yr	138	20 (14.5)	5.48	1.17-25.64	0.03*
	≥4 - <5yr	204	43 (21.1)	8.50	1.89-38.10	0.005*
	≥5 yr	28	9 (32.1)	18.05	3.19-102.01	0.001*
Sex	Female	604	77 (12.8)	0.77	0.35-1.74	0.53
	Male	67	9 (13.4)			
BCS	<2.5	276	44 (15.9)	1.18	0.70-1.91	0.52
	≥2.5	395	42 (11.6)			
Breed	Meat	640	81 (12.7)	0.66	0.22-1.94	0.45
	Milk	31	5 (16.1)			

Discussion

This investigation reveals that age was the only factor shown to be related to the seropositivity against paratuberculosis in mature goats ($p < 0.05$). The results indicate that increasing aged is associated with higher risk of seropositivity for paratuberculosis. The other factors considered, including sex and breed, did not reach a significant level of difference for paratuberculosis infection. Both sexes (female and male) and all breeds (meat and milk) were at the same risk of paratuberculosis infection.

BCS shows no statistically significant difference in the seropositive goats with a BCS lower than 2.5 or equal to and greater than 2.5. In this

investigation, most of the seropositive goat herds considered were small, had proper feeding conditions, and had been established for fewer than two years. These might be the reason why an association between BCS and seropositivity for paratuberculosis could not be detected. Because paratuberculosis is a slow progression of chronic granulomatous enteritis and regional lymphadenitis, infected animals may not show signs of infection for as long as two years, and they may show signs of chronic weight loss, hypoproteinemia, and intermittent diarrhea, resulting in reduced BCS (Mary and David, 2009; Claudia et al., 2016). Clinically affected animals show signs of emaciation, cachexia, and weakness, and death may be

due to secondary infections in the terminal stage of infection (Elizabeth and Michael, 2001).

Based on health and herd management associated with ruminant MAP infections such as presence of other livestock in the herd, introduction of new animals into the herd or movement of animals, and presence of a paratuberculosis testing program, as well as management of kids after birth, such as removing kids from the mother doe immediately after birth and feeding goat kids with colostrum and milk free from MAP, and grouping goats by age, have been reviewed (Eaggel et al., 2014; Gaël et al., 2015; Saray et al., 2015). In this study, many of the farmers are very new goat producers, with established herds of less than two years. These farmers had not known about paratuberculosis in goats and had never tested their herds for this disease. When an animal's health status is unknown to the farmers, the risk of infection becomes high due to transmission of the disease from positive herds to negative herds by selling infected animals or transmission within positive herds due to lack of measures to control the disease. Farmer education on basic knowledge of the disease, such as route of infection and clinical signs, as well as hygienic management practices such as testing paratuberculosis annually in positive herds, maintaining a separate birth area, removing kids from infected mothers immediately after birth, and using a non-mother rearing system, are necessary measures for controlling this disease.

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บทคัดย่อ

ปัจจัยเสี่ยงของความชุกทางซีรัมวิทยาต่อการติดเชื้อพาราทูเบอร์คิวโลซิสในแพะเจริญพันธุ์ เขตภาคตะวันออกเฉียงเหนือของประเทศไทย

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การศึกษาปัจจัยเสี่ยงของความชุกทางซีรัมวิทยาต่อการติดเชื้อพาราทูเบอร์คิวโลซิสในแพะเจริญพันธุ์เขตภาคตะวันออกเฉียงเหนือของประเทศไทยใช้พื้นที่ศึกษาประกอบด้วยจังหวัดบุรีรัมย์ ชัยภูมิ กาฬสินธุ์ ขอนแก่น มหาสารคาม นครราชสีมา ร้อยเอ็ด สกลนคร ศรีสะเกษ และอุดรธานี พบความชุกทางซีรัมวิทยาของแอนติบอดีต่อการติดเชื้อพาราทูเบอร์คิวโลซิสด้วยวิธี ELISA ร้อยละ 12.82 จากแพะทั้งหมด 37 ฟาร์ม จำนวน 671 ตัวอย่าง การเก็บข้อมูลระดับรายตัวจากการตรวจทางคลินิกประกอบด้วยอายุ (แบ่งเป็น 6 กลุ่ม) เพศ คะแนนความสมบูรณ์ของร่างกาย และสายพันธุ์ การวิเคราะห์ด้วย mixed model logistic regression การศึกษาระดับรายตัวพบผลบวกจากการทดสอบด้วย ELISA 86 ใน 671 ตัวอย่าง ปัจจัยเสี่ยงที่พบ ได้แก่ อายุ เมื่อนำแพะที่อายุน้อยกว่า 1 ปีเป็นฐานในการเปรียบเทียบ พบว่าแพะที่อายุมากกว่ามีความเสี่ยงสูงกว่าแพะอายุน้อย กล่าวคือ 3 ปี [($p < 0.05$); OR = 5.48; 95% CI = 1.17-25.64], 4 ปี [($p < 0.01$); OR = 8.55; 95% CI = 1.89-38.10] และมากกว่า 5 ปี [($p < 0.01$); OR = 18.05; 95% CI = 3.20-102.01] ปัจจัยเสี่ยงจากเพศและสายพันธุ์ไม่มีความแตกต่างทางสถิติ โดยสามารถพบได้ทั้งในเพศผู้และเพศเมีย และในทุกสายพันธุ์ ($p > 0.05$) จากปัจจัยเสี่ยงที่พบร่วมกับความชุกทางซีรัมวิทยาของโรคพาราทูเบอร์คิวโลซิสในแพะในภาคตะวันออกเฉียงเหนือของประเทศไทย เกษตรกรควรตระหนักถึงมาตรการในการควบคุมและป้องกันโรคในฝูง เพื่อลดโอกาสการแพร่กระจายโรคและลดความสูญเสียทางเศรษฐกิจ

คำสำคัญ: พาราทูเบอร์คิวโลซิส ELISA แพะ ปัจจัยเสี่ยง ภาคตะวันออกเฉียงเหนือ ไทย

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