เชียงใหม่สัตวแพทยสาร 2560; 15(3): 147-155. DOI: 10.14456/cmvj.2017.14

เชียงใหม่สัตวแพทยสาร

Chiang Mai Veterinary Journal

ISSN; 1685-9502 (print) 2465-4604 (online)

Website; www.vet.cmu.ac.th/cmvj

Original Article

Animal-level factors affecting the results of the first single intradermal test for bovine tuberculosis in dairy calves in Chiang Mai, Thailand.

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Abstract The objective of this study was to identify animal-level factors affecting bovine tuberculosis (bTB) status as determined by the single intradermal tuberculin (SIT) test firstly applied to 1 year-old dairy cattle in Chiang Mai, Thailand. A total of 137 6-month-old calves were randomly selected from 28 small-holder dairy farms in Chiang Mai province, Thailand. Individual data of calves including history of bTB of the farms, bTB status of the dams, general farm management, average dairy gain (ADG) of the calves, occurrence of other diseases, and contacts with other animal species, were collected monthly for 6 months. At ≥ 12 months of age, calves were firstly tested for bTB using the SIT test. Firth penalized logistic regression was used to analyze the associations of factors affecting bTB status of calves. The result showed that more SIT reactors were detected among selected calves from farms with a history of bTB (11.5%, 7/61) compared to those farms with no history of bTB (1.3%, 1/76) ($p < 0.05$). From the logistic regression analysis, we found that calves with high ADG were less likely to become SIT reactors at their first SIT test compared to those with low ADG ($p < 0.01$). To control bTB in dairy cattle, farm practices to limit contact between adult cattle and young calves, together with proper nutritional management for calves are recommended.

Keywords: bovine tuberculosis, animal-level factors, Thailand, single intradermal tuberculin test

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Article history; *received manuscript: 11 August 2017, accepted manuscript: 8 September 2017, published online: 13 September 2017*

บทความต้นฉบับ

ปัจจัยระดับตัวสัตว์ที่ส่งผลต่อการทดสอบวัณโรคด้วยการฉีดแอนติเจนครั้งเดียวทางผิวหนัง ในลูกโคนมในจังหวัดเชียงใหม่ ประเทศไทย

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บทคัดย่อ การศึกษาครั้งนี้มีวัตถุประสงค์ เพื่อหาปัจจัยในระดับตัวสัตว์ที่ส่งผลต่อสถานะของวัณโรคโคในโคนม จากการตรวจด้วยวิธีการทดสอบโรคทางผิวหนังโดยใช้การฉีดแอนติเจนครั้งเดียว ซึ่งปกติจะทำการทดสอบในโคนมที่อายุ 1 ปีขึ้นไป ในจังหวัดเชียงใหม่ ประเทศไทย ลูกโคนมอายุ 6 เดือน จำนวนทั้งหมด 137 ตัวถูกสุ่มเลือกจากฟาร์มโคนมจำนวน 28 ฟาร์มในจังหวัดเชียงใหม่ ประเทศไทย ข้อมูลในระดับตัวโค ได้แก่ ประวัติการพบวัณโรคโคของฟาร์ม สถานะของวัณโรคโคของแม่โค การจัดการลูกโค อัตราการเจริญเติบโตของลูกโค ประวัติการป่วยด้วยโรคอื่นๆ ของลูกโค และการสัมผัสกับสัตว์ชนิดอื่นของลูกโค เป็นต้น เมื่อลูกโคอายุได้ 12 เดือนจึงดำเนินการตรวจวินิจฉัยโรคด้วยวิธีการทดสอบ โรคทางผิวหนังโดยการฉีดแอนติเจนครั้งเดียวทำการวิเคราะห์ข้อมูลโดยการวิเคราะห์ความสัมพันธ์ของปัจจัยที่ส่งผลต่อสถานะวัณโรคโคในลูกโคด้วยสมการถดถอยโลจิสติกแบบวิธีการของเฟิร์ท (Firth penalized logistic regression) ผลของการศึกษาแสดงให้เห็นว่า พบจำนวนลูกโคที่ให้ผลบวกต่อการทดสอบโรคในฟาร์มที่มีประวัติของวัณโรคโคคิดเป็นร้อยละ 11.5 (7/61) ซึ่งมากกว่าในฟาร์มที่ไม่มีประวัติของวัณโรคโคคิดเป็นร้อยละ 1.3 (1/76) อย่างมีนัยสำคัญ ($P < 0.05$) จากการวิเคราะห์ด้วยสมการถดถอยโลจิสติกพบว่าลูกโคที่มีอัตราการเจริญเติบโตสูงมีความน่าจะเป็นที่จะเป็นโคที่ให้ผลบวกต่อการทดสอบโรคครั้งแรก มากกว่าลูกโคที่มีอัตราการเจริญเติบโตต่ำ($P < 0.01$) ดังนั้น การปฏิบัติการในฟาร์มเพื่อจำกัดการสัมผัสระหว่างโคโตเต็มวัยกับลูกโค และมีการจัดการด้านโภชนาการอย่างเหมาะสมสำหรับลูกโค จึงเป็นข้อแนะนำสำคัญเพื่อควบคุมวัณโรคโคในโคนมต่อไป

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Introduction

Bovine tuberculosis (bTB), caused by *Mycobacterium bovis*, is a chronic disease with slowly progressive development of clinical signs (Ayele et al., 2004). Cattle are susceptible to the pathogen. However, it can affect many species of mammals, especially human as a zoonotic disease by inhalation of contaminated aerosols or ingestion of unpasteurized milk (Thoen et al., 1995). Therefore, disease controlling program by detecting and eradicating infected animals has been applied in many countries worldwide to limit the transmission within animals, and between animals and human (Alvarez et al., 2012; Sheridan, 2011).

Various factors at different levels have been identified to be associated with the outbreak of bTB (Humblet et al., 2009). Animal-level risk factors such as age, gender, breed, body condition score, immune status, genetic resistant or susceptible to bTB, vertical and pseudo-vertical transmission and auto contamination, have been previously reported in several countries (Zanini et al., 1998; Phillips et al., 2003; Ozyigit et al., 2007). The age of animals is one of major individual factors that have been identified in both developed and developing countries (Griffin et al., 1996; Cleaveland et al., 2007). The duration of exposure is positively associated with age. The study in Ireland in 1996 found that adult cattle were more likely to be infected animals than younger calves (Griffin et al., 1996). Animals might be infected at a young age and consequently express the clinical signs when they are adults (Griffin et al., 1996). In 2007, a study in Turkey described a congenital infection of bTB from an infected dam to her calf (Ozyigit et al., 2007). Another study suggested that the calves had high risk to be infected with *M. bovis* by ingestion of contaminated colostrum or milk (Zanini et al., 1998). Close contact between a dam and its calf, such as grooming, has been identified as pseudo-vertical transmission (Phillips et al., 2003).

In Thailand, the control program using the single intradermal tuberculin (SIT) test at caudal fold has been implemented to test and eradicate infected dairy cattle annually. The SIT tests apply to adult dairy cattle at the age of 1 year and older. Even though the transmission of *M. bovis* to the young calves may have already occurred in the farm, young animals will not be diagnosed and removed from the farm. Moreover, undetected and infected calves may shed *M. bovis* to healthy cattle before the next eradication program (Kao et al., 2006), which can impede the success of the bTB eradication program.

Therefore, identifying animal-level factors affecting the bTB status of dairy calves based on the results of the first SIT test can be of benefit to the bTB controlling program in the country. Dairy farmers in regions where bTB is regularly detected may be able to prevent and control the transmission of bTB between animals in their farms. The objective of this study was to identify animal-level factors affecting bovine tuberculosis (bTB) status as determined by the single intradermal tuberculin (SIT) test firstly applied to 1-year-old dairy calves in Chiang Mai, Thailand.

Materials and Methods

Study population

A study was performed to identify the animal-level factors associated with bTB in calves to be subsequently identified as bTB reactors. We utilized the bTB status of dairy farms in 4 districts of Chiang Mai province, including Mae Wang, Sanpatong, Doi-Loh and Sansai districts. Infected herds were defined as dairy farms with a history of at least one caudal fold SIT-reactor during 2011 to 2015. Non-infected herds were selected from the population of dairy farms in these districts with no detected or reported SIT-reactor during the same period. Twenty-eight infected and non-infected dairy herds were randomly selected for this study. In each herd, 2-6 calves from 6-8 months of age were randomly selected to be enrolled in the study. A total of 137 calves from these herds were included in the study. All research protocols with animals accorded with protocols approved by the Animal Care and Use Committee, the Laboratory Animal Center, Chiang Mai University, Thailand (Protocol No. 2558/AG-0001).

Data collection

Data including the previous bTB status of the farms, bTB history of dams, general management practices, another disease occurrence, and contacts with other animal species of all calves were collected and monitored monthly until the end of the study. An average daily gain (ADG) was calculated for each animal by recording the weight gain of each calf monthly from the age at enrollment (6-8 months old) until the calf was tested with SIT test (12 months of age).

Single intradermal tuberculin test

At the end of the study, all 137 calves were tested with the caudal fold SIT test using bovine purified protein derivative (PPD) (Bovituber® PPD, Lyon, France). The calves were intradermally injected with 0.1 mL of bovine PPD (2000 IU) on the right side of the caudal fold of the tail. The skin thickness of the inoculation site was measured using a caliper before injection. Test results were determined by the same researcher at 72 h after injection by measuring the increase of skinfold thickness. Interpretations of test results were made according to the Thai agricultural standard for diagnostic testing of bovine tuberculosis (Ministry of Agricultural and Cooperatives, 2004). The results were defined as positive when the increase of the skinfold thickness at the inoculation site was 5 mm or more, or when signs of swelling, edema, exudation, necrosis and inflammation were observed. The results were defined as inconclusive when the increase of the skinfold thickness was between 2-5 mm and clinical signs at the inoculation site were not observed. The results were defined as negative when the skinfold thickness increased less than 2 mm and clinical lesions at the injection site were not observed.

Statistical analysis

Prevalence of bTB among selected calves was calculated in percentages. The ratio of SIT-reactors in herds with previous history of bTB and ratio of SIT-reactors in herds without previous history of bTB were compared using Z-test in R version 3.2.2 (R Core Team, 2016)

According to low animal-level prevalence of bTB reported in this region (Nuamjit and Rodtian, 2012), data were analyzed by Firth type penalized logistic regression which is suitable for analysis of risk factors for rare disease or events. This method corrected the bias from rare events by penalizing the likelihood function (Firth, 1993). The analyses were divided into 2 stages using R version 3.2.2 (R Core Team, 2016). In the first stage, categorical and continuous variables were primarily screened using univariate penalized logistic regressions with the variable of bTB herd status to be analyzed as a random effect. The Firth's method was implemented in R version 3.2.2 (R Core Team, 2016) by the logistf package (Heinze et al., 2016). Evaluation of multicollinearity among predictor variables was assessed using chi-square test for categorical variables ($P < 0.05$) and Pearson product-moment correlation of continuous variables (cor $\geq$ 0.5). In case of multicollinearity (i.e. $P < 0.05$ or cor ≥ 0.5), the variable with higher biological plausibility was retained for multivariate analysis.

After univariate analysis, variables with $P \leq 0.2$ and without marked multicollinearity among the other variables were included in the multivariate penalized logistic regression. A backward selection approach was used for model selection using the logistf package (Heinze et al., 2016) in R version 3.2.2 (R Core Team, 2016).

Results

Descriptive data of enrolled animals

All 137 calves from 28 herds in the study were mixed Holstein-Friesian breed and grown by small-holder dairy farms. Almost half of enrolled calves (61/137, 44.5%) were from infected herds, while another half (76/137, 55.5%) were from non-infected herds. The calves were usually separated from dams at day one after calving and fed with milk twice a day. Most calves (>90%) were not individual raised, and were co-mingled with other calves and/or adult cattle. Most calves were weaned at 3 to 4 months of ages.

Single intradermal tuberculin test

When all calves were ≥ 12 months old and tested with SIT test, 8 out of 137 calves (5.84%) were identified as SIT reactors. Seven out of 8 SIT reactors were from 5 infected herds, while 1 SIT reactor was from a non-infected herd. Animal-level prevalence of bTB from infected herds (7/61, 11.5%) was significantly higher than the animal-level prevalence of bTB from non-infected herds (1/76, 1.3%) ($P < 0.05$).

Factors affecting bTB status of calves

Regarding univariate analysis, 2 variables, including ADG of calves and calves supplemented with trace mineral were significantly associated with the bTB status of calves with $P \leq 0.2$, and no multicollinearity among variables was observed (Table 1). These 2 variables were included in the multivariate logistic regression model by the Firth's method.

In the multivariate logistic regression analysis, the ADG of calves was the only variable remaining in the final model. The ADG of calves was negatively associated with bTB infection in animal-level of dairy calves at $P < 0.01$, as shown in Table 2.

Table 1 Univariate analysis of variables as $P \leq 0.2$ considered for multivariate analysis.

Variable	Mean ± SE or Percentage		Coefficient	P-value
	Positive	Negative		
Average daily gain (ADG in kg/day)	0.50 ± 0.06	0.56 ± 0.02	-2.41	0.0003
Calf was supplemented with trace mineral	60.0%	44.96%	-1.38	0.158

Table 2 Final multiple logistic regression models to find factors affecting bTB in 1-year old calves in Chiang Mai, Thailand

Variable	Coefficient	95% CI	Likelihood ratio test	P -value
ADG	-2.28	-8.97-5.95	6.89	0.009

[#]95% CI = 95% confident interval

Discussion

Cattle herds with a history of bTB are more likely to have a bTB breakdown (Bessell et al., 2012). Moreover, the prevalence of a current bTB in a cattle herd with a history of bTB can be used to predict the severity of future outbreaks (Olea-Popelka et al., 2004). In the current study, we found that the animal-level prevalence of bTB in dairy herds with a history of bTB was significantly higher than that in herds without a history of bTB. Persistent infection of bTB in herds with a history of infection could be associated with the reduced performance of the SIT test, delayed testing, or the reintroduction of infection. The sensitivity of the SIT test has been reported on 75.0–95.5% (de la Rua-Domenech et al., 2006). A lower sensitivity of the SIT test can lead to the failure to detect and remove infected animals, which can potentially create within-herd persistence and onward transmission (Clegg et al., 2011).

In Thailand, the test and eradication program using the SIT test has been implemented to control bTB problem in dairy cattle. The SIT tests are applied to only adult animals at the age of 12 months and older (Nuamjit and Rodtian, 2012). If animals have become SIT-reactors at their first tests, it can be implied that these animals may have been infected with M. bovis before they reach their maturity, in other words, the transmission may have initiated since their calfhood. In general, the management systems for adult cattle and young calves are usually different (Andrews et al., 1991). Certain factors involving the calf raising and some characteristics of the calves may allow or prevent the transmission of M. bovis from infected cows to calves (Humblet et al., 2009).

In the present study, we found that calves with high ADG are less likely to become SIT reactors at their first SIT test, compared to those with low ADG. Several studies also confirm the relationship between body conditions of animals and bTB status. The study in Ethiopia demonstrated that cattle with poor body condition had a higher chance of positive tuberculin test results (Dejene et al., 2016). A cross-sectional study in Zambia suggested that cattle with low body condition score (BCS) had a higher risk of positive SIT results (Cook et al., 1996). A matched case-control study in Ireland suggested that the nutrition factors were associated with the recurrent bTB outbreak because the unbalanced diet of cattle could reduce an animal's resistance to bTB (Griffin et al., 1993). In contrast, the experimental study of the transmission of bTB has reported that dietary restriction did not affect the transmission of the disease (Costello et al., 1998). However, this dietary restriction was performed only in a short duration which may not significantly impair the body condition and the immune status of animals. However, it could not be concluded in the current study that low ADG is the cause or the effect of bTB due to the limitation to confirm the status of M. bovis infection of calves at enrollment. Therefore, the direction of the association between ADG and bTB status in calves should be further investigated.

Conclusion

In this study, we confirmed that transmission of M. bovis to the calves is more common in farms with a history of bTB compared to non-infected farms. Farm practices to limit contacts between adult cattle and young calves should be highly concerned in farms with a history of bTB. Moreover, farmers should focus on management practices of young cattle such as feeding and colostrum management to make young claves getting proper weight gain or staying healthy and to reduce M. bovis exposure to the susceptible young stock, especially on bTB positive farms. However, the number of the SIT positive calves in this study was quite low. Therefore, further study need to be performed in larger populations or larger areas. Moreover, an identification of bTB infection needs to be confirmed by other techniques such as histopathological examination or bacterial culture because SIT test results can be confounded with cross-immune reactions of other mycobacteria. These may provide a further understanding of bTB epidemiology and control. Ultimately, it may help eradicate bTB in Thailaland.

Acknowledgments

The authors would like to thank the Department of Livestock Development of Thailand for providing access to study farms and the World Organization for Animal Health (OIE) Veterinary Educational Twinning Grant as well as the Faculty of Veterinary Medicine, Chiang Mai University and the College of Veterinary Medicine, University of Minnesota for providing partial funding support for this study.

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