

Seroprevalence of melioidosis in goats in southern Thailand and incidence rates of melioidosis sero-positivity in confined and semi-confined goats

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Abstract

This study determined melioidosis seroprevalence in goats in the southernmost provinces known as Thailand's goat dominant areas, and evaluated and compared the incidence rates of melioidosis sero-positivity in confined and semi-confined goats. All goat sera submitted in 2014 under the brucellosis free-farm program at the Veterinary Research and Development Center (Lower southern region) were screened for melioidosis by an indirect haemagglutination test (IHA). Cohorts of 247 confined goats and 927 semi-confined goats were followed up for two years and screened for melioidosis every three months. Any sample with an IHA antibody titre $\geq 1:160$ was defined as melioidosis sero-positive. We observed individual seroprevalence 1.05% (87/8,285); 95% CI 0.85%-1.30%, and herd seroprevalence 7.81% (57/730); 95% CI 6.01%-10.06%. New cases of melioidosis were identified with the incidence rates of 3.24 per day per 100,000 goats in the confined cohort and 7.43 in the semi-confined cohort. Semi-confined goats were 2.29 times more likely to be sero-positive for melioidosis compared with confined goats (incidence rate ratio 2.29; 95%CI 0.91-7.42; $p=0.06$). We concluded that melioidosis seroprevalence prevailed among goat-herds in southern Thailand with low individual prevalence, and confined goats management practices likely reduce melioidosis incidence.

Keywords: goats, melioidosis, seroprevalence, southern Thailand

Introduction

Melioidosis is an infectious disease caused by the gram-negative bacterium, *Burkholderia pseudomallei*. The bacterium is found in soil and water, and is endemic in southeast Asia and tropical Australia (Dance, 1991). Humans and several animal species are susceptible to the infection, which is mainly acquired by ingestion, inhalation and percutaneous inoculation (Sprague and Neubauer, 2004; Wiersinga et al., 2012). Clinical manifestations of melioidosis are non-specific and vary according to the bacterial strain and host immunity (Chen and Currie, 2005). Disease confirmation relies on the isolation and identification of *B. pseudomallei* from clinical specimens (Dance, 1991; Chen and Currie, 2005), however, this method has been shown to have a low sensitivity and is laborious. The most common serological test used to screen for the disease is the indirect haemagglutination (IHA) (Dance, 1991; Chen and Currie, 2005), which is simple and cheap, and can operate on a large scale, but it has a low specificity and therefore needs a confirmation test (Dance, 1991; Cheng et al., 2006). Treatment of melioidosis in animals is not recommended due to high cost, prolonged and often unsuccessful, so that it was mainly managed by preventive and control measures e.g. moving animals from or limits access of animals to contaminated source (Choy et al., 2000).

Thailand has been recognised as an endemic area for melioidosis, with the disease significantly affecting humans and animals nationwide. Incidence rate per 100,000 and case fatality rate of human melioidosis in hyper-endemic areas approached 15.0 and 50%, respectively, and an increasing incidence has been observed (Limmathurotsakul et al., 2010a). The incidence was strongly correlated with prevalence of *B. pseudomallei* in soils (Vuddhakul et al., 1999). A nationwide survey of *B. pseudomallei* reported recovery rates from soil collection sites of 13.8%, 24.5%, 50.1%, and 18.4% in the northern, central, north-eastern and southern regions, respectively (Vuddhakul et al., 1999). Animal melioidosis reports are voluntary and probably underestimate the true incidence due to lack of clinical specimens submitted for laboratory diagnosis. The National Institute of Animal Health and the seven regional veterinary laboratory centers across the country, namely the Veterinary Research and Development Centers (VRDC) have culture-confirmed *B. pseudomallei* in the several domestic and captive wild animals (Limmathurotsakul et al., 2012; Julagiwansujarit et al., 2014). The highest incidence rate was seen in goats, with rates per 100,000 animals during 2006-2010 being 1.63 (Limmathurotsakul et al., 2012). Recent published regional serological surveys of melioidosis in goats have shown a prevalence of 2.76% in the northeastern region (Srikaewkeao

et al., 2008), and 0.19% in the southern region (Chumek and Jeenpun, 2012). Age and sex of goats did not associate with melioidosis occurrence (Chumek and Jeenpun, 2012). Environmental putative risk factors significantly associated with melioidosis in goats were; *B. pseudomallei* present in the soil, farms that have other animal species present, and farms that had flooding or waterlogging conditions (Musa et al., 2015). Management practices that influence the exposure of goats to possible contaminated environment was suggested e.g. raising animals on the floor above the ground (Choy et al., 2000).

As melioidosis affects goats' health and is a zoonotic disease that can spread to humans, monitoring it in the goat population and identifying appropriate preventive measures has been a continued effort to plan for reducing the health risk to livestock and humans. This study determined melioidosis seroprevalence in goats in the southernmost provinces, evaluated incidence rates of melioidosis sero-positivity in confined and semi-confined goats, and determined whether confinement management practice could reduce melioidosis incidence in goats.

Materials and Methods

Study designs and study populations

A cross-sectional study was performed to determine seroprevalence of melioidosis in the goat population of southern Thailand. The study population was goats in the southernmost provinces including Satun, Songkhla, Pattani, Yala, and Narathiwat. The Inclusion criteria were goats that had sera from the entire herd, under brucellosis free-farm program, submitted for laboratory testing between January and December, 2014.

A cohort study was performed for two years, between August 2013 to September 2015, to determine the incidence rates of melioidosis sero-positivity in confined and semi-confined goats. Confined goats were goats that were strictly kept indoor, while semi-confined goats were those that were mainly kept indoor but allow outdoor grazing at certain times. The study population was goats from four convenient-selected farms in southern Thailand.

Data and sample collection

For seroprevalence study, all goat sera subjected to screen for brucellosis under the brucellosis free-farm program and submitted to the Veterinary Research and Development Center (Lower southern region) in Songkhla province, which is the regional veterinary laboratory

centre for the lower southern region, were included for melioidosis test in the present study. Individual data from each goat and their test results were stored in the database at the center.

Cohort of confined goats in two farms and semi-confined goats in other two farms had been screening for melioidosis every three months from August 2013 to September 2015 in order to quickly detect new melioidosis case. The farms were selected purposively because they had individual identification and data of each goat, had practiced routine screening test for melioidosis in goat, and could provide large number of goats to enrol in the study. We collected and stored individual data from each goat and their test results in the designate spread sheet. The end-points of the follow up period for each goat besides those remain and melioidosis-free until the study end were noted: whether the goat was sero-positive for melioidosis, was moved out or died.

Twenty soil samples were collected from each farm where cohorts of goats were followed-up (Limmathurotsakul et al., 2010b). Ten samples each were collected around the goat shelter and in the pasture. The modified fixed-interval sampling strategy was conducted to select the 20 samples. Briefly, to collect the samples around the goat shelter, the first convenient point in a five-metre radius from the goat shelter was selected. Then, the next point to the left, approximately 3.5 metres apart and parallel to the five-metre radius of the goat shelter, was selected and repeated until a total of 10 points were reached. In the pasture, the middle of the pasture was set as the starting point and the next point to the left, approximately 3.5 metres apart and on the same line was selected and repeated for five points. Then, the collector moved forward for 3.5 metres to select other five points parallel to the previous points (Fig. 1).

Evidence of melioidosis occurrence in goats in the four farms before the study (2007-2012) and during the study (2013-2015) was investigated from recorded of culture-confirmed melioidosis in goats diagnosed at the Veterinary Research and Development Center (Upper southern region) in Nakhon Si Thammarat province. We collected the number of goats examined by bacterial culture and the number of culture-confirmed melioidosis goats from each farm over that period.

Laboratory examination

IHA antigen for the melioidosis test was prepared and supplied by the Veterinary Research and Development Center (Upper northern region). Sera were tested for melioidosis by IHA at the Veterinary Research and Development Center (Lower southern region)

(Puapermpoonsiri et al., 1986). Some goat with an antibody titre against *B. pseudomallei* of $\geq 1:160$ was defined as melioidosis sero-positive (NIAH, 2009). A farm with at least one melioidosis sero-positive goat was defined as a positive herd. Soil samples were collected for bacterial culture, in order to identify the presence of *B. pseudomallei*, and submitted to the Faculty of Veterinary Science, Prince of Songkla University where the identification of *B. pseudomallei* was performed using a standard technique (Gilardi, 1985).

Data analysis

Observed individual and herd seroprevalence was reported as the percentage of melioidosis sero-positive cases at the individual and herd levels that were calculated with 95% confidence interval, using Epi Info 6. In addition, odds ratio and Chi-square test were employed to determine if different demographical (type, age, sex) characteristics were associated with melioidosis. The incidence rate is the ratio of the number of melioidosis sero-positive goats to the total time the goats are at risk of disease. Incidence rate was the occurrence of melioidosis sero-positive goats over the total goat-day free of melioidosis (person-time free of event under the study). The incidence rate ratio is the ratio of the two incidence rates, and was calculated with 95% confidence interval, using STATA. The differences were determined based on the Chi-square test ($p \leq 0.05$).

Results

Melioidosis seroprevalence

A total of 8,285 goat sera from 730 herds were screened for melioidosis by IHA. These samples accounted for 4.76% (8,285/173,933) and 2.55% (730/28,574) of the population of goats and goat-herds in the southernmost provinces, respectively (Table 1). The samples were from herds in four provinces; Satun, Songkhla, Yala, and Narathiwat. The herd size ranged from 1-560, with a median herd size of six goats.

Melioidosis sero-positive was identified in 87 goats within 57 herds. An individual seroprevalence was determined to be 1.05% (87/8,285); 95% CI 0.85%-1.30%. Herds that were sero-positive for melioidosis were found in every province, with an overall seroprevalence of 7.81% (57/730); 95% CI 6.01%-10.06%. The seroprevalence at individual and herd level by province were 1.34% (14/1,043) and 13.89% (5/36) in Satun, 0.88% (7/793) and 12.50% (4/32) in Songkhla, 0.10% (2/2,023) and 1.55% (2/129) in Yala and 1.45% (64/4,426) and 8.63% (46/533) in Narathiwat, respectively (Table 1).

Associations between individual characteristics and melioidosis sero-positive

Possible association between melioidosis and three individual demographic characteristics (type, age, and sex) was determined and the findings were shown in Table 2. Dairy goats were 1.58 times more likely to be sero-positive for melioidosis than meat goats, and goats aged over one year were 1.60 times more likely to be sero-positive for melioidosis than those aged ≤ 1 year. Female goats were 1.19 times more likely to be sero-positive for melioidosis than male goats, however, none of these differences were statistically significant ($p>0.05$).

Characteristics of goat farms in the cohort study

Characteristics of goat farms in the cohort study were described (Table 3). A cohort of confined goats was in farm A and B both located in Songkhla province. Farm A used tap water in the farm and had negative results of *B. pseudomallei* in 20 soil examined samples and in all goat specimens submitted for diagnosis at the Veterinary Research and Development Center (Upper southern region) from 2007-2015 (0/19). Farm B used surface water in the farm. It started practiced confined goat management in 2013. *B. pseudomallei* were detected in soil samples in the farm and there were records of *B. pseudomallei* culture-confirmed in goat specimens submitted from farm B during 2007-2012 (8/27). A cohort of semi confined goats was in farm C and D which located in Songkhla and Yala province, respectively. Both farms used surface water. *B. pseudomallei* were detected in soil samples in both farms. There were records of *B. pseudomallei* culture-confirmed in goat specimens submitted from farm C (5/134) during 2012-2015 and in farm D (2/11) during 2007-2012.

Incidence rates of melioidosis sero-positivity in confined and semi-confined goats

Cohorts of 247 confined goats and 927 semi-confined goats were followed up. New cases of sero-positive melioidosis were identified in 5 confined goats and 43 semi-confined goats. The total number of melioidosis-free days among the confined goat cohort and semi-confined cohort were 154,241 and 578,557, respectively. The incidence rates of sero-positive melioidosis per day, per 100,000 goats were 3.24 goats in the confined cohort, and 7.43 goats in semi-confined cohort. Semi-confined goats were 2.29 times more likely to be sero-positive for melioidosis compared with confined goats (incidence rate ratio 2.29; 95%CI 0.91-7.42; $p=0.06$).

Discussion

Overall individual seroprevalence of melioidosis in goats in southern Thailand has been maintained at a low level. It was 1.05% in this study and were 0.40% from the 2002-2003 (Thongnoon et al., 2004) and 1.88% from 2009-2010 (Chumek and Jeenpun, 2012). These were lower when compared with the prevalence of 2.76% (Srikawkheaw et al., 2008) and 7.75% (Naksoontorn and Punurit, 2016) in the north-eastern region, matched the lower prevalence (18.4%) of *B. pseudomallei* in soil in the southern region when compared with that 50.1% in north-eastern region (Vuddhakul et al., 1999). We also detected *B. pseudomallei* in none of 20 soil samples from one farm or only one in twenty of soil samples from other two farms that we investigated. These results should support the low prevalence of *B. pseudomallei* in soil in the study areas. Further, observed low individual and herd seroprevalence of melioidosis in goats in Yala province when compared with the other three provinces could have also associated with possible lower prevalence of *B. pseudomallei* in soils in that province in which no reported cases of human melioidosis were found there from 2004-2015 (BoE, 2004-2015).

The finding of sero-positive goat herds in every province indicating the region-wide presence of *B. pseudomallei* in the region and this supported by the reported cases of human melioidosis in 13 of the 14 provinces in the southern region of Thailand during 2004 to 2015 (BoE, 2004-2015). The overall herd seroprevalence of 7.81% in the current study was greater than the 1.79% seen in the 2009-2010 study (Chumek and Jeenpun, 2012). This could be the result of the power of sample coverage from the higher in number of herds being tested, 730 herds were tested in this study, compared with only 280 herds in the 2009-2010 study. It also could be true increase in incidence as can be associated with an increasing incidence of human melioidosis observed in Thailand during 2006-2015 (personal observation).

We observed no difference in sex, age, or type, whether dairy or meat goats, associated with melioidosis. This finding agreed with the study by Chumek and Jeenpun (2012) but differed from the study by Choldumrongkul (2005) that found melioidosis occurs in male more than female goats, and among goats aged 1 year or more compared to those aged below one year. We assumed even degree of exposure to *B. pseudomallei* among goats of both sex and all age as well as short life span of goat production that limit the influence of putative conditions that increase with age, explained for correspondent incidence among goats of different age and sex.

Melioidosis sero-positive cases were found in confined and semi-confined goats, but the incidence rate was higher in semi-confined goats compared to that of confined goats. With the statistically test for significant lied within the borderline ($p=0.06$), the findings prone to suggest that confinement practice can reduce melioidosis in goats. This was supported by the present of culture-confirmed melioidosis in goats only in the semi-confined goat farms during 2013-2015. Since *B. pseudomallei* habitat is found in the environment, particularly soil and water (Sprague and Neubauer, 2004), adhering to a management practise of only confinement, can prevent goats from extensive exposure to soil, and hence should decrease melioidosis incidence. However, confined management practice alone could not prevent the transmission of *B. pseudomallei* contaminated in water (Inglis et al., 2000), and dust (Chen et al., 2014). Hence, farms in endemic areas should also consider using treated water to feed their animals and adding windshield to animal shelter at time of strong wind.

The limitations of this study arose from the diagnosis based on IHA, and are as follows: The IHA has a low specificity (Cheng et al., 2006), therefore, this study might have included some false positives and overestimated the true incidence of melioidosis in goats. Further, IHA has limited sensitivity in acute septic melioidosis and those cases might have gone undetected (Cheng et al., 2006). Another aspect of limitation from this study was influenced by the study design. A cohort study is suitable when the disease or event is common so that the proper number of cases will be reached for the appropriate analysis in due time. If it is not, the follow up time needs to be extended. This condition is difficult to deal with due to the short production cycle of goats.

Conclusion

In 2014, melioidosis sero-positive prevailed in goat-herds in southern Thailand, and the seroprevalence of melioidosis in individual goats presented has been maintained at a low level. Confined management on goat farms was likely reduce the incidence of melioidosis, as the incidence rate of sero-positive melioidosis was lower than that in semi-confined goats but the difference was not statistically significant at 95% confidence level. Additional measures such as water treatment should be operated on farms in endemic areas to effectively reduce melioidosis in goats.

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Table 1: Seroprevalence of melioidosis in goats in southern Thailand, 2014

Provinces	Goat statistics ^a		Goats			Herd		
	Goats	Herds	Total	Positive (%)	95%CI	Total	Positive (%)	95%CI
Satun	30,235	3,527	1,043	14 (1.34)	0.77-2.30	36	5 (13.89)	4.67-29.50
Songkhla	40,324	4,017	793	7 (0.88)	0.39-1.89	32	4 (12.50)	3.51-28.99
Yala	43,412	8,583	2,023	2 (0.10)	0.02-0.40	129	2 (1.55)	0.19-5.49
Narathiwat	26,727	4,653	4,426	64 (1.45)	1.12-1.85	533	46 (8.63)	6.45-11.42
Total	173,933	28,574	8,285	87 (1.05)	0.85-1.30	730	57 (7.81)	6.01-10.06

^a Source: Department of Livestock Development; Goat statistics in 2014 (DLD, 2014).

Table 2: Demographic characteristics and risk of sero-positive melioidosis in goats in southern Thailand, 2014.

Variables			Sampled goats		%	Odds ratio	p-value
			Total	Positive			
Type	n=7,366	Meat	6,865	70	1.02	1	0.22
		Dairy	501	8	1.60	1.58	
Age	n=5,099	≤1yr	1,598	10	0.63	1	0.18
		>1yrs	3,501	35	1.00	1.60	
Sex	n=8,208	Male	1,643	15	0.91	1	0.55
		Female	6,565	71	1.08	1.19	

Table 3: Characteristics of the goat farms in the cohort study

Cohort of goats	Farms, province	Type of water use	Culture-confirmed <i>B. pseudomallei</i>		
			Soils ^a	Goats	
				Before the study (2007-2012); no.positive/no.sample	During the study (2013-2015); no.positive/no.sample
Confined	A, Songkhla	Tap water	- (0/20)	- (0/11)	- (0/8)
	B ^b , Songkhla	Surface water	+ ^c	+ (8/27)	- (0/15)
Semi-confined	C, Songkhla	Surface water	+(1/20)	- (0/13)	+ (5/134)
	D, Yala	Surface water	+(1/20)	+ (2/11)	- (0/5)

^a 20 soil samples were collected from each farm and examined at the laboratory of faculty of Veterinary Medicine, Prince of Songkla University.

^b Confined management has been applied on the farm from 2013.

^c *B. pseudomallei* detection in soil was confirmed by another research group in 2014.

+/- ; positive/negative from culture-confirmed of *B. pseudomallei* from soil samples examined at the laboratory of faculty of Veterinary Medicine, Prince of Songkla University or goat carcasses or organs submitted for laboratory diagnosis at the Veterinary Research and Development Center (Upper southern region).

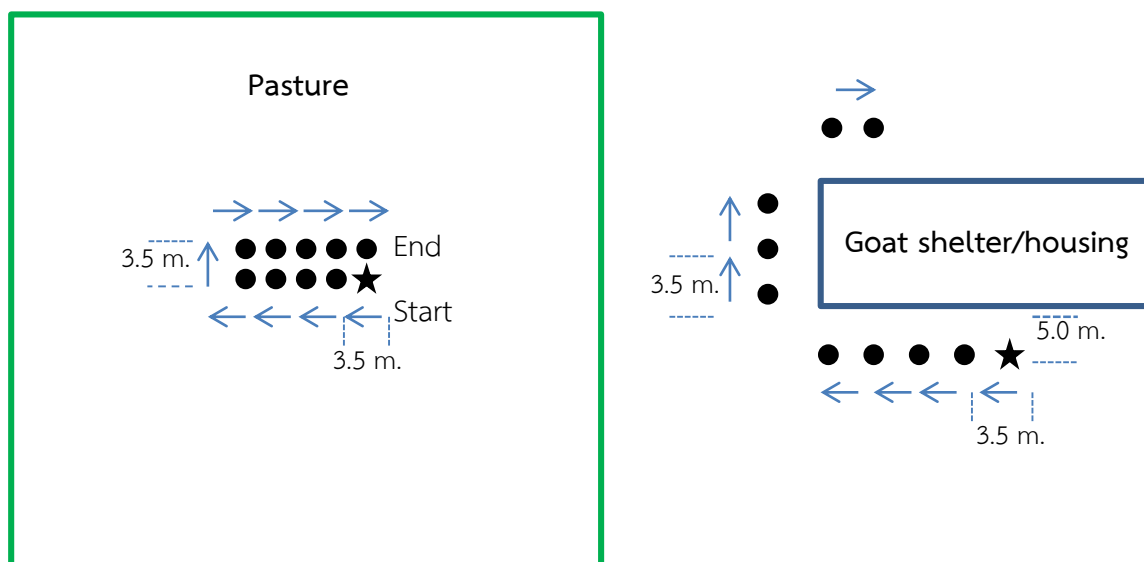


Figure 1. Diagram showed soil collection sites in the pasture (n=10) and around the goat shelter/housing (n=10) aimed to detect the presence of *B. pseudomallei* in the farm environment.

ความชุกทางซีรัมวิทยาของโรคเมลิออยด์ในแพะในภาคใต้ของประเทศไทย และอัตราอุบัติการณ์ของการตรวจพบแอนติบอดีต่อโรคเมลิออยด์ในแพะ ที่เลี้ยงแบบยืนโรงและแบบยืนโรงร่วมกับปล่อยแปลงหญ้า

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บทคัดย่อ

การศึกษาครั้งนี้ทำขึ้นในจังหวัดชายแดนภาคใต้ของประเทศไทยซึ่งเป็นพื้นที่เลี้ยงแพะมากที่สุดของประเทศ วัตถุประสงค์เพื่อประมาณการความชุกทางซีรัมวิทยาของโรคเมลิออยด์ในแพะ ประเมินและเปรียบเทียบอัตราอุบัติการณ์ของการตรวจพบแอนติบอดีต่อโรคเมลิออยด์ในแพะเลี้ยงแบบยืนโรงและแบบยืนโรงร่วมกับปล่อยแปลงหญ้า การทดสอบโรคเมลิออยด์ในแพะด้วยวิธีอินโดเรกซิมแอกกลูติเนชัน ของห้องปฏิบัติการศูนย์วิจัยและพัฒนาการสัตวแพทย์ภาคใต้ตอนล่าง ใน พ.ศ.2557 และติดตามกลุ่มแพะเลี้ยงแบบยืนโรง 247 ตัว เลี้ยงแบบยืนโรงร่วมกับปล่อยแปลงหญ้า 927 ตัว ทดสอบโรคเมลิออยด์ทุก 3 เดือน เป็นเวลาประมาณ 2 ปี ตัวอย่างที่มีระดับแอนติบอดี $\geq 1:160$ จัดว่าให้ผลบวก ผลการศึกษาพบความชุกทางซีรัมวิทยาในระดับรายตัว 1.05% (87/8,285); 95% CI 0.85%-1.30% ความชุกในระดับฝูง 7.81% (57/730); 95% CI 6.01%-10.06% อัตราอุบัติการณ์ของการตรวจพบแอนติบอดีต่อโรคเมลิออยด์ต่อวันต่อแพะ 100,000 ตัว เท่ากับ 3.24 ในแพะเลี้ยงแบบยืนโรง และ 7.43 ในแพะเลี้ยงแบบยืนโรงร่วมกับปล่อยแปลงหญ้า ซึ่งแพะเลี้ยงแบบยืนโรงร่วมกับปล่อยแปลงหญ้ามียาอัตราอุบัติการณ์การตรวจพบแอนติบอดีต่อโรคเมลิออยด์เป็น 2.29 เท่าของแพะเลี้ยงแบบยืนโรง (incidence rate ratio 2.29; 95%CI 0.91-7.42; $p=0.06$) โดยสรุป พบความชุกทางซีรัมวิทยาของโรคเมลิออยด์แพร่หลายในฝูงแพะในภาคใต้แต่มีความชุกรายตัวที่ระดับต่ำ อัตราอุบัติการณ์ของโรคที่สูงกว่าในกลุ่มแพะเลี้ยงแบบยืนโรงร่วมกับปล่อยแปลงหญ้าบ่งชี้ไปในทิศทางที่กล่าวได้ว่าเลี้ยงแพะแบบยืนโรงลดอุบัติการณ์ของโรคเมลิออยด์ได้

คำสำคัญ: แพะ, โรคเมลิออยด์, ความชุกทางซีรัมวิทยา, ภาคใต้ของประเทศไทย