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Molecular Detection of *Leptospira* Infection in Meat Goat of Southern, Thailand

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ABSTRACT

Leptospirosis is a serious zoonotic disease causes by *Leptospira* spp. infection, and Thailand is an endemic area for both humans and animals. Infected animal such as cattle, sheep and goats can mostly be asymptomatic but can experience foetal abortions and stillbirths. Infected animals play a role in spreading the bacteria via urine. This study determined leptospirosis in meat goats of Southern Thailand by a molecular detection method. In total, 323 serum samples were collected from meat goats from representative provinces of Southern Thailand, namely Songkhla (179) and Yala (144), from April to June 2022 by the veterinarian of the Regional Livestock Health Unit 9, Songkhla, Thailand. Antibodies from previous infections were screened using the lepto-latex test. Polymerase chain reaction (PCR) targeted the *LipL32* gene, and overall positivity was 76.47% (247/322). There was no statistically significant difference between the results from Songkhla (76.54%) and Yala (76.39%). The *LipL32* PCR indicated an overall positivity of 9.29% (30/323), positive samples from Yala (18.75%) were significantly more abundant than those from Songkhla (1.68%) (P<0.00001). This study indicates a low active infection with a high previous infection (seroprevalence) of leptospirosis in meat goats, suggesting the circulation of leptospirosis of meat goats in Southern Thailand. DNA sequencing for analysing the serovars distribution among goat is needed. Disease prevention via vaccination based on serovar in Thailand be considered. Moreover, molecular detection of leptospirosis be applied for surveillance at herd level, and farmers and persons in contact with goats should be aware of *Leptospira* infections.

Key words: Molecular detection, Leptospirosis, Meat Goat, Southern Thailand.

INTRODUCTION

Leptospirosis causes foetal abortions and stillbirths in cattle, sheep and goats. This pathogen is spread by urine from infected animals, contaminating pastures, water and feed. Infected animals can carry the bacteria for a long time and may not display any clinical signs. Goats are more susceptible to *Leptospira* than other ruminants and more frequently experience abortions as a result of the infection. Acute or chronic infections go along with common symptoms such as anaemia, icterus, hemoglobinemia and fever; in some cases, icterus can be absent (Monahan et al. 2009). According to previous studies, hematobiochemical changes in infected goats indicate hepatic damage (Vihol et al. 2019).

Leptospirosis is a global zoonosis and considered as a workplace health and safety issue for farmers, especially in tropical and subtropical regions including Thailand. In humans, infection is mostly caused by exposure to infected animals or urine-contaminated soil or water. In Thailand, during the period from 2003–2018, the annual incidence rate ranged from 3.1–6.6 per 100,000 inhabitants (Hinjoy 2014; Chadsuthi et al. 2017). The seroprevalence of human leptospirosis was 23.7% (Suwancharoen et al. 2013). From 2007–2008, the overall nationwide leptospirosis IgG seroprevalence among young men entering the Royal Thai Army was %28 (Gonwong et al. 2017). High-risk areas of human leptospirosis are mostly the lower north-eastern and southern parts of Thailand, associated with population density, elevation, and rice cultivation areas (Chadsuthi et al. 2017). The highest risk of human leptospirosis associated with rainfall was observed in the northern and north-eastern regions (Phosri 2022).

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In Thailand, the seroprevalence values of leptospirosis recorded in ruminants during January to August 2001 such as cattle, buffaloes, sheep and goats were 9.9, 30.5, 4.7 and 7.9%, respectively (Suwancharoen et al. 2013). In another study, seroprevalence in buffaloes and cattle during 2010-2015 was 24.8 and 28.1%, respectively (Chadsuthi et al. 2017). In Southern Thailand, the seroprevalence of leptospirosis in bullfighting was 27% and molecular detection was 13% (Ngasaman et al. 2022).

However, there are no reports of leptospirosis of meat goats in Southern Thailand. In this context, the present study investigated the molecular prevalence of leptospirosis in meat goats from two provinces in the upper (Songkhla) and lower part (Yala) of Southern Thailand to elucidate the status of leptospirosis in meat goat population. Moreover, to estimate the risk of transmission to the human.

MATERIALS AND METHODS

Ethics approval

This study has been approved by the Institutional Animal Care and Use Committee, Prince of Songkla University, Thailand (Approval No. MHESI 68014/33).

Study design and sample size calculation

This study was designed to be conducted in two provinces representative of the upper and lower parts of Southern Thailand, namely Songkhla and Yala. The required sample size was calculated using the Scalex SP calculator (Naing et al. 2022) for the expected prevalence of 7.9% (Suwancharoen et al. 2013) and was 311 for the margin of error or absolute precision of ±3% in estimating the prevalence with a 95% confidence. With this sample size, the anticipated 95% CI positive result was (4.9%, 10.9%).

Study areas and sample collection

The veterinarian of the Health Unit of Regional Livestock 9 collected 323 serum samples of meat goats from April to June 2022 in the two provinces. The blood samples form Songkhla and Yala were 179 and 144 respectively. All animals were not vaccinated against *Leptospira*. The samples were sent to the laboratory of the Faculty of Veterinary Science, Prince of Songkla University, Thailand., and 200µL of serum was used for DNA extraction according to the manufacturer´s instruction (Geneaid™ DNA Isolation Kit, Taiwan).

Laboratory diagnosis

The samples were screened by a lepto-latex test developed by the Department of Medical Sciences, Ministry of Public Health. Briefly, the serum was equally mixed with *L. interrogans* Pyrogens antigen on a glass slide and gently mixed with a sterile tip; and agglutination was observed after 2–5 minutes by the naked eye. Subsequently, *LipL32* PCR for *Leptospira* was applied as previously described by Romero-Vivas et al. (2013) .The PCR reaction mix included 5.0µL of Taq polymerase master mix (KAPA®, Japan), 0.25µL of each primer, 4.5µL of ultra-pure water and 2.0µL of DNA template, with a total volume of 12µL. The PCR cycle consisted of an initial denaturation step at 95°C for 5 min, followed by 35 cycles of 1 min at 94°C for denaturation, 1 min at 60°C for annealing and 1 min at 72°C for extension, with a final extension at 72°C for 7 min. The PCR products were electrophoresed on 1.5% agarose gels at 100 V for 30 min, and the identified fragments had a size of 423 base pairs.

Data analysis

The proportion and 95% confidence intervals of positive results by the screening test and *LipL32* PCR were analysed using an online free website (https://sample-size.net). Differentiation of positive results between the two tests (lepto-latex test and *LipL32* PCR) as well as between the two provinces (Yala and Songkhla) was performed using a free online website (https://www.socscistatistics.com/).

RESULTS

Total serum samples were obtained from 323 meat goats from the provinces of Songkhla (179) and Yala (144). Overall positivity by the lepto-latex test was 76.47% (227/323) The result by the province indicated the seropositive by MAT in Songkhla 76.54% (110/144) and in Yala 76.39% (137/179), but it was not significantly different (P>0.05) (Table 1, Fig. 1).

The PCR showed an overall positivity of 9.29% (30/323) (95% CI: 6.35–12.99). The positivity by PCR in Yala (18.75%) was higher than Sonkhla (1.68%). This difference was statistically significant (P<0.00001) (Table 2, Fig. 1). However, the positive results from the screening test (Lepto latex test) were not significantly related to the positive by PCR for both provinces (P>0.05).

In Yala Province, PCR positive samples (27) were from 3 seronegative and 24 seropositive by MAT. Of the positive samples by PCR form Songkhla Province, 1 and 2 samples were from seronegative and seropositive, respectively. However, the number of PCR-positive samples which from the seronegative and seropositive by MAT of both provinces did not significantly differ (P-values 0.089 and 0.68, respectively).

Table 1: The results of Lepto-latex test in meat goats of Songkhla and Yala provinces

Province	Total samples	No. of Lepto-latex test results		% positive	95% CI
		Negative	Positive		
Yala	144	34	110	76.39	68.60–83.06
Songkhla	179	42	137	76.54	69.64–82.54
Total	322	76	247	76.71	71.70-81.22

Table 2: The results of PCR detection in meat goats of Songkhla and Yala provinces

Province	Total samples	No. of PCR detection		% positive	95% CI
		Negative	Positive		
Yala	144	117	27	18.75*	12.73–26.10
Songkhla	179	176	3	1.68*	0.35–4.82
Total	323	293	30	9.29	6.35–12.99

* Significant difference (P<0.00001)

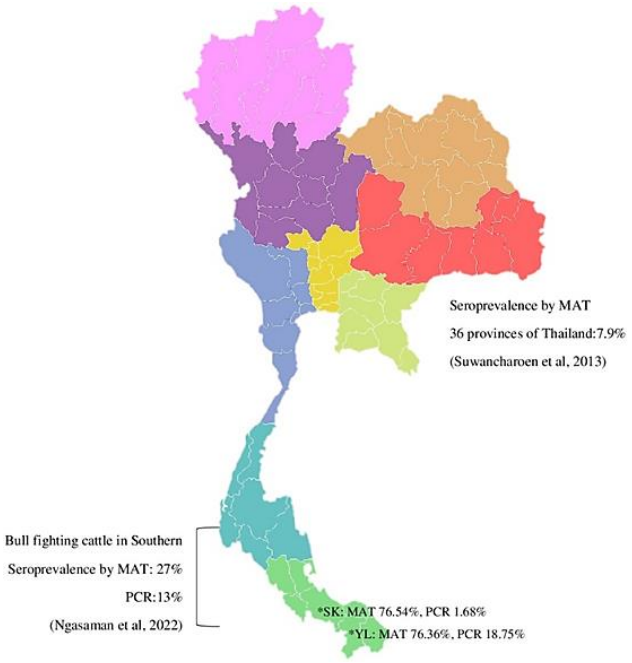

Fig. 1: Molecular prevalence of leptospirosis in meat goat in Southernmost Thailand in this study compared with the results of the previous study in ruminants of other region of Thailand.

DISCUSSION

We observed a high positivity (76.47%) when applying the lepto-latex test in meat goats. We did not use the gold standard for serology diagnosis (microscopic agglutination test; MAT) as serovar cultivation is time-consuming and costly. However, the lepto-latex test can be used for screening prior to the PCR, and the results can be observed within 5–10 minutes.

Previous studies from Thailand using MAT demonstrated a low seroprevalence in sheep (4.7%) and goats (7.9%) (Suwancharoen et al. 2013). However, in other ruminants from Southern Thailand, seroprevalence was high (27%) in cattle used for bullfighting (Ngasaman et al. 2022), similar to the seroprevalence in cattle from Northern Thailand (31.76%) (Rojanasthien et al. 2005). In neighbouring countries such as Malaysia, goats and sheep showed a seroprevalence of 11.20% and 5.03%, respectively (Sabri Abdul Rahman et al. 2020). A meta-analysis by Zaki et al. (2020) indicated the following seroprevalence values of *Leptospira* spp. in animal reservoirs in Southeast Asia: sheep and goats 8.17%, rodents 17.95%, pigs 19.24%, large ruminants 24.90%, carnivores 27.28%.

A previous study of leptospirosis Thailand was reported in human and other species of livestock. The most commonly detected antibodies in sheep and goats of Thailand were those against Mini, Shermani and Ranarum (Suwancharoen et al. 2013). While, the most predominant serovars in human and livestock in Thailand were Shermani, followed by Bratislava, Panama, and Sejroe in human, Shermani, Ranarum, and Tarassovi in buffaloes, and Shermani and Ranarum in cattle and pigs (Chadsuthi et al. 2022).

The most common serovars of *Leptospira* involved in caprine abortion were Grippotyphosa, Pomona, Icterohaemorrhagiae and Autumnalis (Loureiro and Lilenbaum 2020). However, the serovars found in goats were not the cause of symptomatic leptospirosis, and these animals may be asymptomatic during infection but can spread the pathogen to the environment. Interestingly, a study in Nan Province, using rrs nested PCR, identified *Leptospira* in 9.92% of human urine samples and water samples from livestock areas. In goats, *L. intergans* could not be detected, whereas *L. weilii*, which has not previously been identified in Thailand, was recovered. This sequence type is closely related to previous isolates from cases of human leptospirosis in Laos and China (Kurilung et al. 2017). Furthermore, serovar infections in meat goats in Southern Thailand should be investigated to determine potential relationships with human infections.

There are few studies on the molecular detection of leptospirosis in meat goats in Southern Thailand. This study identified a low active infection rate (9.29%), indicating that infected goats can transmit *Leptospira* spp. to other animals on the same farm as wells as to the farmers and slaughterhouse workers. Three samples from Yala and one sample from Songkhla were negative by the lepto-latex test and positive by the PCR test. This indicates that these goats were in the early infection phase, below 8 days. Typically, leptospirosis follows a biphasic course; the first stage, the septicaemic phase, lasts for 4–7 days, and during this phase, leptospires can be found in the blood and cerebrospinal fluid. In the second stage, the immune phase, *Leptospira* antibodies begin to be produced, and the bacteria are excreted in the urine (Fraga et al. 2011). In a study from Iran, nested PCR targeting the 16S rRNA gene detected pathogenic *Leptospira* in goat urine (0.9%) and in sheep urine (4.87%) of asymptomatic, which indicates that these animals can excrete *L. interrogans* through urine, transmitting it to other animals and humans (Haji Hajikolaei et al. 2022).

In the present study, samples from Yala showed higher active infection than those from Songkhla, although they were collected in the same season. This can be explained by the different farming styles; whilst goat farms in Yala are small, and the goats are kept on ground, in Songkla, goat farming is more intense. Generally, farmers in Yala feed their animals home-grown roughage such as grass from rubber plantations, palm plantations, leaves and vegetable scraps. In contrast, farmers in Songkhla feed their animals acacia leaves, which grow at a greater height than grass. Therefore, the feed source might be one of risk factors for *Leptospira* infection in meat goats. However, there are several other potential risk factors of caprine leptospirosis, such as flooding (Sabri et al. 2019), animal management, hygienic status of the farm, herd size, animal age, previous history of abortion and access of rodents to the farm (Desa et al. 2021)

In the present study, the previous leptospirosis infection rates in meat goats from Sothern Thailand were high, whereas the current infection rates were low. This indicates that previously infected animals shed the pathogen into the environment, which is then transmitted to other animals. Moreover, meat goats in Southern Thailand are a potential source of pathogenic *Leptospira* spp. for human leptospirosis. The molecular detection of pathogenic *Leptospira* should be applied for surveillance in the herd as well as for health checks during quarantine prior

to the introduction of new goats into the herds, and vaccination should be considered. Public awareness of leptospirosis infection in people who are in contact with goats should be strengthened.

Conclusion

This research is the first time for molecular detection of leptospirosis infection in meat goat in Southern of Thailand. The result indicated that an active infection of leptospirosis was not high even seroprevalence was high. Moreover, molecular detection method can be used to determine an early infection that can treated the infected goat and prevent transmission to human.

Conflict of interest

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organization related to the material discussed in the manuscript.

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