Veterinary Integrative Sciences 2022; 20(1): 85 - 93. DOI; 10.12982/VIS.2022.008

Vet Integr Sci

Veterinary Integrative Sciences

ISSN; 2629-9968 (online)

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

Research article

The study of prevalence and factors affecting *Anaplasma maginale* infection in domestic goats in Chonburi province, Thailand

Thanakrit Chankong[1], Donruthai Srita[1], Pongphon Tongsangiam[2], Sirilak Meesuwan[1] and Kanoknaphat Klinpakdee[1,*]

[1]Faculty of Veterinary Medicine, Rajamangala University of Technology Tawan-ok, Chonburi 20110, Thailand
[2]Veterinarian at Livestock Hospital, Faculty of Veterinary Medicine, Rajamangala University of Technology Tawan-ok, Bang Phra campus, Chonburi 20110, Thailand

Abstract

The objective of this study was to determine the prevalence and factors affecting *Anaplasma marginale* infection of goat farming in Chonburi province, Thailand. A total of 182 samples were collected from clinical services records from the Veterinary Teaching Hospital, Rajamangala University of Technology Tawan-ok. Moreover, the potential risk factors of *A. marginale* infection, including vectors infestation, barn-types, preventive medicine protocols using vector-control agents and/or deworming programs, and the farming areas were statistically analyzed. The results revealed that 24.7% of goats were positive for *A. marginale* infection. The Nhong Yai district had the highest infection rate at 34.1% (15/44). The Mueng, Chonburi and Sri Racha district goats were infected at the rate of 27.5% (11/40) and 24.3% (9/37), respectively. The potential significant factors affecting *A. marginale* infection were vectors infestation ($P < 0.01$) and preventive medicine protocols using vector-control agents and/or deworming programs ($P < 0.05$). In conclusion, the vectors infestation increases the chance of *A. marginale* infection. Conversely, appropriate consistent preventive medical health management by application of vector-control agents and/or deworming programs can reduce the likelihood of infection. Our study could serve as a guideline for prevention planning and for further study of livestock rearing behavior concerning infection of blood parasites in nearby areas.

Keywords: *Anaplasma marginale*, Goats, Prevalence, Risk factors, Chonburi province

Corresponding author: Kanoknaphat Klinpakdee, Faculty of Veterinary Medicine, Rajamangala University of Technology Tawan-ok, 43 Moo 6 Bangphra, Sriracha, Chonburi 20110, Thailand. Tel: +66 38358137, Fax: +66 38358141, e-mail: kanokwan_si@rmutto.ac.th

Funding; National Research Council of Thailand and Rajamangala University of Technology Tawan-ok, 2018

Article history; *received manuscript: 21 Sebtember 2021, revised manuscript: 5 October 2021, accepted manuscript: 17 November 2021, published online: 23 November 2021*
Academic editor; *Korakot Nganvongpanit*

Open Access Copyright: ©2022 Author (s). This is an open access article distributed under the term of the Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution, and reproduction in any medium or format, as long as you give appropriate credit to the original author (s) and the source.

INTRODUCTION

Goat farming in Thailand has been increasing during the last decades. The reasons for its popularity include easy husbandry, yield in both meat and milk, taking up a little space, quick breeding, rapid capital return, and low risk of investment. Goat farming can also be a supplementary occupation, along with other main jobs in the form of household farms or business farms like other main occupations (DLD, 2007).

The Department of Livestock Division (DLD), Thailand reported that the amount of goat production in Chonburi province had increased to at least 2,000 animals in 2012, most of which were goats produced for meat. Previously, cattle production was only the main existing ruminant production in this area. The Chonburi Provincial Livestock office also reported that the number of farm-raised goats and sheep had reached 2,500 animals by 2014 and then gradually increased to more than 6,000 animals by 2019 (DLD, 2020b). However, data from the Veterinary Research and Development Center, Eastern Region (VRD-EP) in 2020 demonstrated that the goat population in Chonburi was gaunt, anemic and some had died, the main cause of which was infestation from external - internal parasites or blood protozoa infection (DLD, 2020a).

There are several types of blood protozoa in goats, including *Babesia* spp., *Theileiria* spp., *Anaplasma spp.*, *Trypanosoma evansi*, and Plasmodium. Moreover, some of these blood parasites are associated with multi-species livestock hosts such as the *Trypanosoma evansi* infection in cattle as well as swine and the *Babesia* ovis infection in both sheep and goats. The infected animals show common clinical signs, including exhaustion, emaciation, pale mucous membrane, low appetite, subcutaneous edema, weight loss, anemia, decreased immunity, and loss in productivity such as stunted growth, low feed efficiency and decreased dairy production (Sahinduran, 2012).

One of the most important blood parasites in goats is *Anaplasma marginale*. Its morphology is rounded measuring 0.3 – 1 µm in diameter. The cell is purple - black in color according to Wright's Giemsa stain and usually found at the margin site of red blood cells (Stoltsz, 1993). This blood protozoa causes Anaplasmosis resulting in illness and death in ruminants, including cattle, buffaloes, goats, and sheep (Howden et al., 2010; Kocan et al., 2010, 2003). Infection is usually caused by ticks or blood-sucking insects such as mosquitoes or tabanid flies. In addition, idiopathic infection caused by injection, castration, or surgical operation cannot be ruled out (Chomel et al., 1994). The common clinical signs are high fever at around 39.5-41.2° C, anorexia, emaciation, severe anemia, panting, as well as pale mucous membranes. In Thailand, Anaplasmosis is considered as one of the most frequent diseases causing death in ruminants. The drug of choice for treatment in ruminants is oxytetracycline 20 mg/kg by deep intramuscular injection twice a week and/or combined with imidocarb dipropionate (Imizol®) 2.2 mg/kg once every two weeks, along with blood tonic and fluid therapy (Sahinduran, 2012). The outbreak can be controlled by culling or separating sick animals out of the herd, using a vector-control agent every 2-3 months and farm disinfection, especially for equipment and tools (Howden et al., 2010).

According to the DLD, Anaplasmosis has been reported in cattle, buffaloes, goats, and sheep during the 2014-2019 time period in Livestock Administrative Region 2, which includes the following provinces: Chanthaburi, Chachoengsao, Chonburi, Trat, Nakhon Nayok, Nakhon Sawan, Prachinburi, Rayong, Samutprakarn, and Sa Kaeo. There were 70 samples of Anaplasmosis infection in ruminants (cattle, buffaloes, goats and sheep) from 2014 to 2019, comprising 31 samples in Sa Kaew (44.29%), 22 samples in Chonburi (31.43%), 12 samples in Chanthaburi (17.14%), 2 samples in Nakhon Nayok (2.86%), 2 samples in Prachinburi (2.86%), and 1 sample in Trat (DLD, 2020a)

A. marginale infection in goats results in economic losses for goat farmers. They receive less income from reduced productivity but have to incur more expenditure for sick goats' treatment and lose more profits from fatal cases. It has been reported that Chonburi has a large number of goats with *A. marginale* infections, which do not include subclinical or undifferentiated para-clinical-sign cases. These unrecorded cases would still be a hidden cost in goat production as well as a burden for farmers. Thus, the objective of this study was to identify the prevalence and factors affecting *A. marginale* infection in domestic goats in Chonburi province. The research team was interested to detect this protozoa by using blood smears and microscopic findings according to the standard protocol recommended by the OIE (2008). The data obtained from our study could provide an important baseline information for epidemiological study, which benefits prevention planning in various animals and giving proper advice to goat farmers for disease control and prevention.

MATERIALS AND METHODS

Sample selection

From the clinical services record of *A.marginale* infection in goats by livestock unit, the Veterinary Teaching Hospital, Rajamangala University of Technology Tawan-ok from October 2019 to September 2020, the researchers chose five districts of Chonburi which the most frequency of A.marginale infection in goats was found and selected all 'goat cases' in these five districts as samples, consisting of Mueng, Chonburi (40 samples), Sri Racha (37 samples), Ban Bueng (26 samples), Koh Chan (35 samples), and Nong Yai (44 samples). Then, farmers' phone numbers and addresses were recorded to facilitate farm visits.

Owing to blood sample collection and laboratory diagnostic protocol in the Veterinary Teaching Hospital, Rajamangala University of Technology Tawan-ok, clinicians have collected 3 ml of blood sample from the jugular vein and placed in an EDTA coated blood sampling tube, then tested for *A. marginale* using the thin blood smear technique dying of Diff – Quick stain protocol (Stoltsz, 1993) and determined the infection using microscopic inspection.

Data Collection

Data collected through farm visits, interviews, interview forms, and farm surveys consisted of vectors infestation (non-infested and infested), barn-types (platformed and grounded), regular preventive medicine protocol implementation: using the vector-control agent and/or deworming by applying protocols every 3 months (vector-control agents and deworming through regular use, vector-control agents only through regular use, vector-control agents and deworming through non-regular use, vector-control agents only through non- regular use, deworming only through regular use, deworming only through non-regular use, and never using an agent), and farming areas (rural, agricultural field/ natural vegetation).

Statistical analysis

The correlation between *A. marginale* and the relevant risk factors was analyzed using univariable and multivariable logistic regression with the R statistical software version 6.4.1 (R Core Team, Vienna Austria). Initially, for univariable analysis, the chi-square test was performed to determine the relationship between *A. marginale* infection and the relevant risk factors. Factors with significance levels of P-value ≤ 0.2 from the univariable analysis were selected for multivariable logistic regression analysis. In the event of multicollinearity ($P < 0.05$), the factors with higher biological acceptability were retained for multivariable logistic regression analysis by Stepwise backward selection using P-value as a stopping rule (Hosmer and Lemeshow, 2000).

Ethical Approval

The authors confirm that the ethical policies of the journal, as noted on the journal's guideline page, have been adhered to and the appropriate ethical review committee approval has been received. The Animal Ethics Committee of Rajamangala University of Technology Tawan-ok, Chonburi, Thailand has reviewed and approved this study (RMUTTO-ACUC-2-2021-018)

RESULTS

A total of 182 samples were included in this study at the following distribution values: 21.98% in Mueng, Chonburi ($n = 40$), 20.33% in Sri Racha ($n = 37$), 14.29% in Ban Bueng ($n = 26$), 19.23% in Koh Chan ($n = 35$), and 24.18% in Nhong Yai ($n = 44$). Most of the samples were raised in ground-barns (68.13%), non-infested by vectors (52.75%) and most of the farming areas (90.11%) were agricultural fields or natural vegetation. Regarding the preventive medicine protocol, 35.16% of the samples had regular vector-control agents and deworming, 18.68% had regular vector-control agents only, 6.04% never used an agent, and other descriptive data are shown in Table 1.

Table 1 Findings for districts, vectors infestation, barn types, preventive medicine protocols, farming areas and *A. marginale* infection

Items	Percentages (Observation numbers/ total numbers)
Districts	
Mueng, Chonburi	21.98% (40/182)
Sri Racha	20.33% (37/182)
Ban Bueng	14.29% (26/182)
Koh Chan	19.23% (35/182)
Nhong Yai	24.18% (44/182)
Vectors infestation	
Non-infested	52.75% (96/182)
Infested	47.25% (86/182)
Barn-types	
Ground	68.13% (124/182)
Platform	31.87% (58/182)
Preventive Medicine Protocols	
-Never use an agent	6.04% (11/182)
-Vector-control agents only: by regular use	18.68% (34/182
- Vector-control agents and deworming: by non-regular use	13.19% (24/182)
-Vector-control agents only: by non-regular use	10.44% (19/182)
-Deworming only: by regular use	8.79% (16/182)
-Deworming only: by non- regular use	7.69% (14/182)
- Vector-control agents and deworming: by regular use	35.16% (64/182)
Farming Areas	
Rural	9.89% (18/182)
Agricultural field/ Natural Vegetation	90.11% (164/182)
A. marginale infection	
Positive	24.7% (45/182)
Negative	75.3% (137/182)

A comparison of the *A. marginale* infection data showed that there was a positive reading for 45 samples out of a total 182 samples (24.7%), while the Nhong Yai district had the highest infection rate at 34.1% (15/44). In the Mueng, Chonburi and Sri Racha districts, goats were infected at the rate of 27.5% (11/40) and 24.3% (9/37), respectively. There were two significant factors acquired from the univariable analysis with P-value < 0.05. However, with the criteria, P < 0.2, 3 the factors that were included in the model selection process for multivariable analysis included vectors infestation, preventive medicine protocols, and farming areas (Table 2), by Stepwise backward selection using P-value, the final model included two factors: 'vectors infestation' and 'preventive medicine protocol' (Table 3).

Table 2 Prevalence of *A. marginale* infection with variables base on univariable logistic regression analysis (P-value < 0.2)

Variables	Observation Quantities	*A. marginale* infection quantities (Percentage)	X^2	Df	P-values
Districts					
Mueng, Chonburi	40	11 (27.5)			
Sri Racha	37	9 (24.3)			
Ban Bueng	26	4 (15.4)			
Koh Chan	35	6 (17.1)			
Nhong Yai	44	15 (34.1)	4.542	4	0.338
Vectors infestation					
Non-infested	96	4 (4.2)			
Infested	86	41 (47.7)	46.136	1	< 0.01
Barn-types					
Ground	124	29 (23.4)			
Platform	58	16 (27.6)	0.374	1	0.541
Preventive Medicine Protocols					
-Never use an agent	11	6 (54.5)			
-Vector-control agents only: by regular use	34	3 (8.8)			
- Vector-control agents and deworming: by -non-regular use	24	13 (54.2)			
-Vector control agents only: by non-regular use	19	8 (42.1)			
-Deworming only: by regular use	16	6 (37.5)			
-Deworming only: by non- regular use	14	6 (42.9)			
- Vector-control agents and deworming: by regular use	64	3 (4.7)	41.818	6	< 0.01
Farming Areas					
Rural	18	7 (38.9)			
Agricultural field/ Natural Vegetation	164	38(23.2)	2.153	1	0.142
Total	182	45 (24.7)			

Table 3 Risk factors from the final logistics regression model for *A. marginale* infection in domestic goats in Chonburi, Thailand

Risk Factors	Odds Ratios	95% CI	P-values
Preventive Medicine Protocols			
-Never use an agent (reference group)			
-Vector-control agents only by regular use	0.103	0.016 - 0.645	0.015
- Vector-control agents and deworming by non- regular use	0.827	0.164 - 4.164	0.818
-Vector-control agents only by non-regular use	0.524	0.098 - 2.812	0.451
- Deworming only by regular use	0.630	0.106 - 3.742	0.611
-Deworming only by non-regular use	0.572	0.096 - 3.420	0.541
-Vector-control agents and deworming by regular use	0.095	0.015 - 0.585	0.011
Vectors infestation	11.936	3.788 – 37.609	< 0.01

DISCUSSION

The aim of this study was to determine the prevalence and factors affecting *A. marginale* in domestic goats in Chonburi Province. The prevalence of *A. marginale* in domestic goats in our study was 24.7 % (45/182). This value might be higher than the true prevalence because the data was collected from the clinical services record based on goat farm visits requested by the farmers who need the services from livestock unit, the Veterinary Teaching Hospital, Rajamangala University of Technology Tawan-ok. It might infer about an outbreak of *A. marginale* in those farms previously. In comparison, according to the previous study conducted by Kaewchana et al. (2021), the prevalence of *Anaplasma spp.* of bullfighting cattle in southern of Thailand was 0.10% (2/1906). In the other studies, *A. marginale* is endemic among cattle in Peninsula, Malaysia with 72.6% prevalence (Ola-Fadunsin et al., 2018) and the prevalence of *A. marginale* of cattle in Salakpra Wildlife Santuary, Kanchanaburi province was 54.1% (132/244) (Saetiew et al., 2015)

A consideration of the relationship between risk factors and the detection of *A. marginale* led to the conclusion that vectors infestation increased the likelihood of infection by nearly 12 times compared to non-infestation (3.788 – 37.609 at 95% CI). Concerning the preventive medicine protocol with 'never use an agent' as a control group, regular use vector-control agents and deworming reduced *A. marginale* infection risk by 90.5% (41.5 - 98.5% at 95% CI), and only use vectors-control agents regularly reduced risk by 89.7% (35.5 – 98.4% at 95% CI) with statistical significance ($p = 0.011$ and 0.015 respectively). Even if the vectors-control agents were provided but not used regularly, this did not produce a statistically significant difference compared to 'never use an agent', which correlates with the results of a study by Ola-Fadunsin et al (2018). They claimed that management systems, the presence of ticks and frequency of de-ticking were the risk factors significantly associated ($P < 0.05$) with the detection of *A. marginale* in cattle. In addition, the use of deworming drugs did not affect the reduction of the risk of *A. marginale* infection. Based on observation by giving only deworming drugs,

whether consistent or not, there was no statistical difference comparing to 'never use an agent', which was in accordance with a study by Saetiew et al. (2015). They found that deworming was not significantly associated with detection of *A. marginale* ($P < 0.05$).

Turning to consider the factors that contained no significant differences, such as the "barn-types" or "farming areas", most of the goat farmers in Chonburi province release their goats onto the ground, pasture, or nearby areas in order to make it easier for them to clean the stalls, together with letting goats to roam freely in order to reduce stress and decrease the amount of feed/water that farmers have to provide in the stalls. Particularly, result of 'the farming areas' in our study contradicted the study of Kasozi et al. (2021) that small ruminants located at the forest edge (<0.3 km) showed higher prevalence of *A. ovis* infection than those found inland or midland regions associated with increased agricultural activity. Additionally, the samples of goats being raised in platformed-barns at all times should be included in further study.

CONCLUSIONS

This research concluded that while vector-infested goat farms suffer from increased risk of *A. marginale*, preventive medicine protocol with proper and consistent vector-control agents can reduce the risk of infection. However, the uneven or inappropriate protocol showed no difference compared to non-protocol. Our finding could guide the planning of disease prevention, as well as serving as a baseline for further study of farming habits and infections in other livestock nearby in the future.

ACKNOWLEDGEMENTS

The success of this study was made possible through the support of the National Research Council of Thailand and Rajamangala University of Technology Tawan-ok, by funding the budget expenditure (on income) for the fiscal year 2018. The Chonburi Provincial Livestock Office supported the research by providing livestock breeding data from 2014 to 2020 and the Veterinary Research and Development Center, Eastern Region, provided epidemiological data relating to the *Anaplasma marginale* from 2014 to 2019. Furthermore, the Veterinary Teaching Hospital, Rajamangala University of Technology Tawan-ok, provided clinical data and samples profiles. Finally, 6th Grade Veterinary Students in the Academic Year 2020, assisted with sample collection and laboratory analysis.

AUTHOR CONTRIBUTIONS

PT, TC, KK selected and collected the samples.
PT, SM, KK collected and categorized the data.
TC, DS conceived and designed the analysis.
TC, DS, SM analyzed the data.
TC, KK designed the art work and tables.
TC, KK, SM wrote the manuscript.

REFERENCES

Chomel, B.B., Carniciu, M.L., Kasten, R.W., Castelli, P.M., Work, T.M., Jessup, D.A. 1994. Antibody prevalence of eight ruminant infectious disease in California mule and black tailed deer (*Odocoileus hemionus*). J. Wildl. Dis. 30: 51-59.

Department of Livestock Development. 2007. Goat Farming, 2nd ed.; Division of Livestock Extension and Development. Bangkok, Thailand. pp. 1-2. (in Thai)

Department of Livestock Development. 2012. Summary of data and statistics number of goats and farmers for the year 2011. Information and Communication Technology Center. Retrieved from https://ict.dld.go.th/webnew/index.php/th/service-ict/report/365-statsummary-typeanimal/statsummary-goat (in Thai)

Department of Livestock Development. 2020. Epidemiological Data of *Anaplasma marginale*. Veterinary Research and Development Center, Eastern Region. Retrieved from http://docimage.dld.go.th/dldform2/Home5e/mainfrm.asp?m=bf (in Thai)

Department of Livestock Development. 2020. Summary of data and statistics number of goats and farmers for the year 2019. Information and Communication Technology Center. Retrieved from https://ict.dld.go.th/webnew/index.php/th/service-ict/report/365-statsummary-typeanimal/statsummary-goat (in Thai)

Hosmer, D.W., Lemeshow, S. 2000. Applied Logistic Regression, 2nd ed.; Cressie, N.A.C., Fisher, N.I., Eds.; John Wiley & Sons, Inc.: New York, NY, USA; pp. 143–202.

Howden, K.J., Geale, D.W., Paré, J., Golsteyn-Thomas, E.J., Gajadhar, A.A. 2010. An update on bovine Anaplasmosis (*A. marginale*) in Canada. Can. Vet. J. 51(8): 837-840.

Kaewchana, N., Rakwong, P., Ngasamani, R. 2021. Haemoparasites infection in bullfighting cattle in southern of Thailand. Vet. Interg. Sci.19(2): 133-140.

Kasozi, K.I., Welburn, S.C., Bahita, G.E., Marraiki, N., Nalumenya, D.P., Namayanja, M., Matama, K., Zalwango, K.K., Matovu, W., Zirintunda, G., Ekou, J., Kembabazi, S., Mugasa, C.M., Kitibwa, A., Tayebwa, D.S., Musinguzi, S.P., Mahero, M., Ssengendo, I., MacLeod, E.T. 2021. Molecular epidemiology of anaplasmosis in small ruminants along a human-livestock-wildlife interface in Uganda. Heliyon, 7(1). e05688.

Kocan, K.M., de la Fuente, J., Guglielmone, A.A., Meléndez, R.D. 2003. Antigen and alternative for control *A. marginale* infection in cattle. Clin. Microbiol. Rev. 16(4): 798-712.

Kocan, K.M., de la Fuente, J., Blouin, E.F., Coetzee, J.F., Ewing, S.A. 2010. The natural history of *A. marginale*. Vet. Parasitol. 167(2-4): 95-107.

OIE (World Organization for Animal Health). 2008. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, 6th ed.; OIE Standards Commission.: Paris, France; pp. 601–602.

Ola-Fadunsin, S.D., Gimba, F.I., Abdullah, D.A., Sharma, R.S.K., Abdullah, F.J.F., Sani, R.A. 2018. Epidemiology and risk factors associated with *Anaplasma marginale* infection of Cattle in Peninsular Malaysia. Parasitol. Int. 67(6): 659-665.

Saetiew, N., Simking, P., Yatbantoong, N., Ninphet, W., Laosomboon, P., Pumrojana, P., Jittapalapong, S. 2015. Prevalence and risk factors of *Anaplasma marginale* infections in beef cattle in Salakpra Wildlife Sanctuary, Kanchanaburi Province. J. Mahanakorn Vet. Med. 10(2): 69-80.

Sahinduran, S. 2012. Protozoan Diseases in Farm Ruminants, A Bird's-Eye View of Veterinary Medicine, Dr. Carlos C. Perez-Marin (Ed.). IntechOpen, DOI: 10.5772/30251. Available from: https://www.intechopen.com/chapters/28686.

Stoltsz, W.H. 1993. Bovine Anaplasmosis. In current Veterinary Therapy 4: Food Animal practice, J.W. Howard (ed). W.B. Sauder Company, Philadelphia, PA, USA; pp. 588-596.

How to cite this article;

Thanakrit Chankong, Donruthai Srita, Pongphon Tongsangiam, Sirilak Meesuwan and Kanoknaphat Klinpakdee. The study of prevalence and factors affecting *Anaplasma maginale* infection in domestic goats in Chonburi province, Thailand. Veterinary Integrative Sciences. 2022; 20(1): 85-93.