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Research article

Toxoplasma gondii and *Neospora caninum* prevalence and risk factors on goat farms in Kanchanaburi province, Thailand

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Abstract

Toxoplasma gondii and *Neospora caninum* are apicomplexan protozoan parasites that have been associated with reproductive problems in ruminants. Despite the high seroprevalence of *T. gondii* and *N. caninum* reported in goats worldwide, little information about the seroprevalence of these parasites in goats in Thailand. In this study, we investigated the seroprevalence of *T. gondii* and *N. caninum* in 389 goat samples from five districts of Kanchanaburi province, Thailand. An indirect enzyme-linked immunosorbent assay (iELISA) using recombinant TgGRA7 and NcSAG1 antigens was used for the detection of anti-*T. gondii* and anti-*N. caninum* antibodies, respectively. Risk factors for the occurrence of these protozoan parasites on goat farms were also assessed. Specific IgG against *T. gondii* and *N. caninum* was detected in 28.5% and 16.7% samples, respectively. Co-infections were observed in 11.8% of samples. The risk factors significantly associated with *T. gondii* seroprevalence was the presence of cats (odds ratio [OR]= 2.55, 95% confidential interval [CI]=1.68- 3.89). The presence of other domestic animals on farm contributed to the risk of *T. gondii* and *N. caninum* infection on the goat farms (OR=1.70, 95% CI=1.09- 2.64).

Keywords: Goat, *Neospora caninum*, Risk factor, Seroprevalence, *Toxoplasma gondii*, Thailand

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INTRODUCTION

Toxoplasma gondii and *Neospora caninum* are cyst-forming apicomplexan coccidian protozoan parasites that are distributed worldwide. Toxoplasmosis is one of the most common zoonoses, affecting almost all endothermic animals, including humans. Approximately one-third of the global human's population has been infected with *T. gondii* (Montoya and Liesenfeld, 2004). Toxoplasmosis causes significant economic losses for sheep and goat farming industry; it can induce abortion, stillbirth, congenital malformations and neonatal losses (Tenter et al., 2000). Neosporosis caused by *N. caninum*, which is recognized to be one of the most common pathogens causing abortion in cattle, sheep, goats, buffalo, and camels (Dubey and Schares, 2011). *N. caninum* is closely related to *T. gondii*, but the former has not been confirmed as a zoonosis. However, low antibody titers to *N. caninum* have been reported in humans (Robert-Gangneux and Klein, 2009).

The serological prevalence of *T. gondii* and *N. caninum* in animals has been examined worldwide (Tenter et al., 2000; Dubey, 2003). Among farm animals in Thailand, the seroprevalence of *T. gondii* in beef cattle was found to be 15.2% using an indirect fluorescent antibody technique (IFAT) or 25.7% using an enzyme-linked immunosorbent assay (ELISA) based on dense granule antigen 7 (TgGRA7) (Wiengcharoen et al., 2012; Udonsom et al., 2018). The seroprevalence was 9.4% or 17% in dairy cattle, when a latex agglutination test (LAT) or ELISA was used, respectively (Inpankaew et al., 2010), and 27.9% in domestic goats when LAT was used (Jittapalapong et al., 2005). Seroprevalence studies of neosporosis in farm animals in different parts of Thailand have been carried out. The prevalence of this infection varies from 9.7% to 64% in cattle (Wiengcharoen et al., 2012; Inpankaew et al., 2014; Udonsom et al., 2018), and from 2.8% to 16.7% in buffalo (Nam et al., 2012; Kengradomkij et al., 2015). In Thailand, goats are raised in an extensive or semi- extensive farming system. The goat farms are enclosures located in farmer's house. In this farming system, the animals are contacted with dog and cat that could be exposed to *T. gondii* and *N. caninum* infections. However, no data are available for its prevalence in goats.

Considering the scarcity of information available about the occurrence of *T. gondii* and *N. caninum* infections in goats in Thailand, this study aimed to investigate the frequency of antibodies against these parasites in sera from goats in Kanchanaburi province, Thailand, using an indirect enzyme-linked immunosorbent assay (iELISA) with specific recombinant antigens. In addition, a risk factor analysis was performed to evaluate the risks associated with these parasites in the goat farms, with the aim of identifying effective prevention and control strategies for these protozoans on goat farms.

MATERIALS and METHODS

Ethical approval and informed consent

All procedures that involved animals were approved by the Animal Care and Use Committee of the Faculty of Veterinary Science, Mahidol University, Thailand (Approved No. MUVS-2018-06-31). Informed consent was obtained from all goat farmers involved.

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Study area

Kanchanaburi is the largest province in western Thailand, with an area of approximately 19,483 km². The province is divided into thirteen districts. According to Thailand's Department of Livestock Development record in 2019 there were 54,281 goats in Kanchanaburi province. Most goats (99.33%) in this area are raised for meat (Department of Livestock Development, 2019).

Animals and samples

A cross-sectional study design was used. The study was conducted from June 2018 to May 2019. Sample size was determined based on an assumed prevalence of 27.9% (Jittapalapong et al., 2005) and was calculated with 95% confidence at an absolute precision of 5% assuming random sampling. Individual sampling points were sampled at 95% confidence with test sensitivity 90%. using online server (https://epitools.ausvet.com.au/ freedom). Therefore, at least 10 animals were randomly selected from each farm. A total of 389 blood samples, from 19 goat farms, were collected from randomly selected herds from five districts in Kanchanaburi province. Goat farms were selected among those officially registered in the veterinary health care center, Faculty of Veterinary Science, Mahidol University. Blood samples were collected from the jugular vein and were immediately transferred into 10 ml vacuum blood tubes without anticoagulant. The sera were separated and stored at −20 °C until examination.

Data regarding the characteristics of animal and herd management were obtained via questionnaires administered by the investigators. Data items collected included the age, sex and breed of individual animals. Farm-based characteristics recorded included the location, herd size (small to medium: 10 to 30, or large herd size: greater than 30 goats), water supply (ground-water or tap water), farm management (intensive or semi-intensive), presence of cats (yes/no), presence of dogs (yes/no) and presence of other domestic animals on the farm (yes/no).

Indirect ELISA (iELISA)

Recombinant TgGRA7 and NcSAG1 were expressed from dense granule proteins of *T. gondii* and from surface antigens of *N. caninum*, respectively, as previously described (Chahan et al., 2003; Terkawi et al., 2013). The purified recombinant protein of TgGRA7 and NcSAG1 fused with glutathione S-transferase was prepared and used as the antigen.

The protocol of iELISA was performed as previously described (Fereig et al., 2016) with slight modifications. Purified protein (100 μL) at a final concentration of 0.1 μg was coated onto MaxiSorp plates (Nunc, Roskilde, Denmark) overnight at 4 °C in a coating buffer (50 mM carbonate/bicarbonate buffer, pH 9.6). The plates were washed five times with phosphate-buffered saline (PBS) containing 0.01% Tween 20 (PBS/Tween) and blocked with 5% PBS-skimmed milk (PBS-SM) at 37 °C for 1 hour. The plates were washed five times with PBS/Tween, and 50 μL goat serum diluted 1:250 with PBS-SM was added to each well. The microplates were incubated for 1 hour at 37 °C. After washing, 50 μL horseradish-peroxidase-conjugated anti-goat IgG antibodies (Invitrogen, CA, USA) diluted 1:5000 with PBS were added. After incubation

for 1 hour at 37 °C followed by washing, the color was developed by the addition of a substrate 3,3',5,5'-tetramethylbenzidine (Invitrogen, CA, USA); the reaction was then stopped by the addition of 0.1 M HCl. The optical density (OD) was measured at 450 nm using a microplate reader (model ELx808, Biotex, VT, USA). ELISA results were determined in duplicate for each serum sample.

The cut-off point for the OD value of a positive sample was set as the mean value of standard *T. gondii*- or *N. caninum*-negative control goat sera plus five standard deviations (Fereig et al., 2016). The negative and positive control sera were confirmed by MAST® TOXOREAGENT (Mast Group, Liverpool, UK) for *T. gondii* and IFAT for *N. caninum*.

Statistical analysis

The data analysis was performed using SPSS version 25.0 software for Windows (SPSS Inc., IL, USA). The seroprevalence of *T. gondii* and *N. caninum* infections was calculated for all samples. Two categorical variables, sex and breed were excluded due to the high proportion of female and Boer goat in this study. Univariable analysis was performed using the Chi-square test or Fisher's exact test to determine the association between the presence of the seropositivity and exposure variables. Variables with a P-value <0.05 were retained and selected for a multivariate logistic regression model performed by a stepwise backward elimination. Model fit was assessed using the Hosmer-Lemeshow test (P-value >0.05).

RESULTS

Demographic characteristics of the study population

Of the 389 goats sampled, from 19 farms, 269 (69.1%), 50 (12.9%), 30 (7.7%), 30 (7.7%) and 10 (2.6%) were from Sai Yok, Thong Pha Phum, Phanom Thuan, Bo Phloi and Muang districts of Kanchanaburi province, respectively (Figure 1). The majority of animals (77.6%) were age between 1 and 3 years, the remainder were aged more than 3 years old. Females accounted for 98.97% of the goats sampled. Most of the goats were raised for meat (97.2%), with the rest farmed for daily purpose. The dominant breed in this study (82%) was the Boer goat. Most (61.4%) of the farms were small to medium and keep between 10 to 30 heads of goat. Most goats (68.4%) were reared in a semi-intensive farming system. Thirteen (68.4%) farms had dogs and eight farms had cats (42.1%). Furthermore, 64% of farms kept other domestic animals (such as cattle, sheep and chickens) in close proximity to their goats. Tap water (65.8%) was the major source of water used on the farms.

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Figure 1 Location map of the sampling sites in Kanchanaburi province, Thailand (blue star). The numbers refer to the districts investigated in this study (1=Sai Yok, 2=Thong Pha Phum, 3=Phanom Thuan, 4=Bo Phloi and 5=Muang).

Serology

All of goat farms were seropositivity at least one parasite infection. The overall seroprevalence of *T. gondii* and *N. caninum* was 28.5% and 16.7%, respectively. The seroprevalence of *T. gondii* and *N. caninum* co-infections was 11.8%. According to locality, the prevalence of *T. gondii* infection was as follows: 30.1%, 20%, 46.7%, 13.3% and 20% were recorded for Saiyok, Thong Pha Phum, Phanom Thuan, Bo Phloi and Muang, respectively. The positive samples of *N. caninum* infection were detected in Saiyok 15.2%, Thong Pha Phum 16%, Phanom Thuan 36.6%, Bo Phloi 10% and Muang 20%, localities (Table 1).

Table 1 Seropositivity of *T. gondii*, *N. caninum* and co-infections with these organisms among goats in Kanchanaburi province, Thailand.

Location	No. of herds	Total no. of goats	Number of goat IgG antibody positive (%)		
			T. gondii	*N. caninum*	Co-infections
Saiyok	15	269	81(30.1)	41 (15.2)	36 (13.4)
Thong Pha Phum	1	50	10 (20)	8 (16)	3 (6)
Phanom Thuan	1	30	14 (46.7)	11 (36.7)	4 (13.3)
Bo Phloi	1	30	4 (13.3)	3 (10)	3 (10)
Muang	1	10	2 (20)	2 (20)	0
Total	19	389	111 (28.5)	65 (16.7)	46 (11.8)

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Risk factor analysis

To examine the predisposing factors for *T. gondii* and *N. caninum* infections, ages of animals, herd size, water supply, farming system, presence of cats or dogs, and presence of other domestic animals on the farm were analyzed. Univariable analysis revealed that the presence of *T. gondii* infection was statistical significantly associated with the presence of cats on a farm (P<0.001). The presence of other animal species on a farm had an association with *T. gondii* (P=0.002) and *N. caninum* (P=0.004) infection on those farms (Table 2). There were no significant differences (P>0.05) in the seroprevalence of these parasites among different ages, herd size, farming systems, water supply types or the presence of dogs. The multivariable analysis results (Table 3) indicated that *T. gondii* seroprevalence in goats was mainly influenced by the presence of cats on a farm (odd ratio [OR]=2.55, confidence interval [CI]=1.67- 3.89) and the presence of other domestic animals on a farm (OR=1.69, CI=1.08- 2.63).

DISCUSSION

Although the seroprevalence of *T. gondii* and *N. caninum* has been reported in goats around the world, investigations of the infection rates of these parasites in goats from Thailand are limited. We therefore performed a study of the seroprevalence of *T. gondii* and *N. caninum* on goat farms in Kanchanaburi province, Thailand. In this study, we used an indirect ELISA with specific recombinant antigen for *T. gondii* (dense granule protein, TgGRA7) and *N. caninum* (tachyzoite, NcSAG1), which has previously been used, both by others researchers and also by our working group to investigate specific antibodies against *T. gondii* and *N. caninum* in various animal species (Fereig et al., 2016; Ichikawa-Seki et al., 2016; Udonsom et al., 2018). The infection rate of *T. gondii* (28.5%) in this study is consistent with previous epidemiological studies in Satun province, Thailand that showed 27.9% prevalence (Jittapalapong et al., 2005). In comparison with other countries, the prevalence of *T. gondii* found in the present study was lower than in Bangladesh (61%) (Rahman et al., 2014), India (41.3%) (Singh et al., 2015), and China (29.5%) (Liu et al., 2015). The differences in seroprevalence rates seen in these countries could be due to differences in serological techniques, the number of animals tested, and climatic and farm management variations. The seroprevalence of *N. caninum* (16.7%) was lower than *T. gondii* in the present study. However, the prevalence rate of *N. caninum* in goats was higher than the previously reported prevalence of 8% in beef cattle (Udonsom et al., 2018) and 15% in dairy cattle sera (Arunvipas et al., 2012) in the same area. This indicated a wide distribution of *N. caninum* infection among goats in Kanchanaburi province. However, to fully determine the current situation of infection with *N. caninum* in goats in Thailand, a large-scale study with an increased number of farms and animal samples from more provinces should be undertaken.

Table 2 Univariable analysis of risk factors associated with *T. gondii* and *N. caninum* infections.

Variables	*T. gondii*				*N. caninum*			
	No. tested	No. positive	OR (95% CI)	P-value (<0.05)	No. tested	No. positive	OR (95% CI)	P-value (<0.05)
1. Ages								
Less than 1 year	41	24	2.29	0.35	27	7	0.99	0.98
1-3 years	270	103	(0.37-3.94)		198	58	(0.44-1.39)	
2. Herd size								
Small to medium	171	66	1.15	0.56	109	25	1.59	0.11
Large	107	45	(0.70-1.88)		116	40	(0.89-2.85)	
3. Water supply								
Tap water	189	69	1.55	0.09	150	40	1.39	0.58
Ground mater	89	42	(0.93-2.59)		75	28	(0.88-2.20)	
4. Farm management								
Semi-intensive	266	110	1.14	0.55	149	46	1.34	0.36
Intensive	123	47	(0.73-1.76)		76	19	(0.71-2.50)	
5. Presence of cats								
Yes	110	61	2.93	<0.001	87	29	1.55	0.06
No	168	50	(1.78-4.84)		138	36	(0.99-2.42)	
6. Presence of dogs								
Yes	163	66	1.48	0.06	125	45	1.355	0.193
No	115	35	(0.97-2.26)		100	20	(0.85-2.14)	
7. Presence of other domestic animals on farm								
Yes	167	79	2.21	0.002	148	52	2.66	0.004
No	111	32	(1.33-3.69)		77	13	(1.33-5.29)	

OR=odd ratio, CI=confidence interval

Table 3 Multivariable logistic regression analysis of risk factors associated with *T. gondii* infection in goats.

Category	Adjusted OR	95% CI	P-value (<0.05)
1. Presence of cats on farm			
Yes	2.55	1.68 -3.89	<0.001
No	Ref		
2. Presence of other domestic animals			
Yes	1.70	1.09 -2.64	0.002
No	Ref		

OR=odd ratio, CI=confidence interval

Analysis for putative risk factor revealed that the seropositive rate for *T. gondii* among goats raised on farms where cats were present was approximately 2.5- times higher than that for animals raised on farms without cats. This finding is also consistent with a previous report on goat farms in China, which reported that the odds of the presence of *T. gondii* antibodies increased 3.2-fold if cats were present on farms (Liu et al., 2015). Cats act as the definitive host in the life cycle of *T. gondii*, excreting oocysts in their faces that can be a source of infection (Dubey, 1998). Therefore, the association with the presence of cats is assumed to indicate a causal relationship that may increase the possibility of infection from final hosts to intermediate hosts. Previous risk factor studies have found the presence of dogs on farms to be a putative risk factor for *N. caninum* seropositivity in goats (Topazio et al., 2014; Liu et al., 2015). However, the presence of dogs was not identified as a potential risk factor in this study. A reason for this variable not being a risk factor in our study might be that farming areas are inaccessible to pet dogs. A high seropositivity for *N. caninum* among goat samples was observed in our study area; therefore, further studies to investigate other risk factors may help to further clarify our findings.

The universal risk for infection with *T. gondii* or *N. caninum* was the presence of other animal species. Most of the farms selected in this study (64 %) had various different animal species (such as cattle, sheep and chickens) living in the same environment. However, it is unclear; how the presence of other animal species plays a role in the transmission of these parasites at a farm level. One study of toxoplasmosis on goat farms in Myanmar suggested that the cohabitation of different animal species and the movement of these animals could facilitate the spread of the infection (Bawm et al., 2016). Therefore, studies on the relationship between goats and other domestic species that maintain *T. gondii* and *N. caninum* infections in the herd is necessary to better understand the epidemiology of these pathogens among goat farms in Thailand.

CONCLUSION

In summary, the results of our study revealed that *T. gondii* and *N. caninum* infections are widely distributed among goats in Kanchanaburi province, Thailand. The presence of cats on a farm was clearly associated with a higher odds ratio for being seropositive for *T. gondii* in goats. In addition, the presence of other domestic animals was a risk for the occurrence of *T. gondii* and *N. caninum* infections among goats in this area.

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AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: CJ. Performed the experiments: RU, JS, OT, KN. Analyzed the data: CJ. Contributed reagents, materials, and analysis tools: RMF, YN. Project supervisor: YN. Wrote the manuscript: RU, CJ. All authors read and approved the final manuscript.

CONFLICT of INTEREST

The authors declare that they have on conflict of interests.

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