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Full Length Research Paper

Seroprevalence and risk factors influenced transmission of *Toxoplasma gondii* in dogs and cats in dairy farms in Western Thailand

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The objectives of this study were to determine seroprevalence of *Toxoplasma gondii* infection in dogs and cats in dairy farms of the western provinces of Thailand, and to evaluate the risk factors for the infection. Dogs and cats from positive dairy farms were collected as case and pets from neighboring farms were randomly selected as control; 40 herds in total. 114 dogs and 36 cats sera sample was tested in the Western provinces, and examined for antibodies against *T. gondii* infections by latex agglutination test. Seven sera samples from dogs (6.1%, 6/114) and 3 sera samples from cats (8.3%, 3/36) were found to have titer of *T. gondii* ranging from 1:64 to 1:256 in dogs and from 1:256 to 1:2048 in cats. A toxoplasmosis-positive herd with the presence of a pet was tended to have a 4.6 times (OR = 4.6, 95%CI: 0.95, 22.5; P = 0.06) higher chance of fecal contamination of *T. gondii* infections compared to a herd without the presence of pets. In herds with positive pets on the farm, the prevalence was 6.9 times (OR = 6.9, 95% CI; 1.06-50.8; P = 0.01) more likely to have positive cows in the herd.

Key words: *Toxoplasma gondii*, seroprevalence, risk factors, dogs, cats.

INRODUCTION

Toxoplasma gondii is a ubiquitous protozoan parasite of warm-blooded animals (Dubey, 2010). It has an extremely broad host range including birds, wild animals, pets, livestock and humans. *T. gondii* is prevalent in most areas of the world. Environmental contamination with *T. gondii* oocysts extends even into the oceans. High *T. gondii* seroprevalence rates were recently demonstrated in a variety of marine mammals (Dubey, 2004). *T. gondii* is of veterinary and medical importance, because it may cause abortion or congenital disease in its intermediate hosts. It is estimated to infect 30% of the population worldwide (Flegr and Striz, 2011). Infections in most immunologically normal humans are asymptomatic or result in an influenza-like illness, which often goes undiagnosed as Toxoplasmosis (Tenter et al., 2000). Women who become infected for the first time during pregnancy and immunosuppressed patients are at serious risk from toxoplasmosis. *Toxoplasma* infection can be life threatening in congenitally infected and immunosuppressed patients (Chintana et al., 1998). *T. gondii* is recognised worldwide as a major cause of morbidity and mortality in AIDS patients, primarily as a result of encephalitis (Luft et al., 1984). While *T. gondii* can be transmitted directly by animal-human contact or through contact with contaminated feces, soil and herbage, it can also be transmitted through contaminated food and water. Humans usually become infected with *T. gondii* by ingesting tissue cysts from undercooked meat

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or by ingesting food or water contaminated with oocystes from infected cat feces (Kijlstra and Jongert, 2008). In animals, *T. gondii* infection not only results in significant reproductive and hence economic losses, but also has implications for public health since consumption of infected meat or milk can facilitate zoonotic transmission (Hill et al., 2005).

Cats play an important role in the spread of toxoplasmosis as they are the only animals that excrete resistant oocysts into the environment (Dubey, 2006). On farms this can result in significant reproductive problems in dairy cows (Arunvipas et al., 2012) and economic losses in livestock from abortion and neonatal mortalities (Dubey, 2010).

The standard diagnosis of toxoplasmosis recommended by the OIE for cats is based on coprological diagnosis. The results are often difficult to interpret since the numbers of oocysts of *T. gondii* in cat feces are usually low (Dubey and Beattie, 1988). The latex agglutination test is now widely available as a useful tool for the serological diagnosis of toxoplasmosis and was used by this study as detecting antibodies ensured more accurate results. Serological surveys are good indicators of the occurrence of *T. gondii* infection in cats which is important as serologically positive cats undoubtedly shed oocysts (Dubey and Thulliez, 1989).

Serological studies in Thailand have found evidence of widespread in humans with 1.2 to 4.6% (Maruyama et al., 2000), cats with 11% and dogs with 9.4% (Jittapalapong et al., 2007), goats with 27.9% (Jittapalapong et al., 2005), dairy cows with 7% (Arunvipas et al., 2012). The overall seroprevalence of *T. gondii* was found in 21 of 136 (15.4%) captive felids (Thiangtum et al., 2006), and elephants with 25.6% (Tuntasuvan et al., 2001). There is no evidence on the association between dogs and cats within dairy farms in Thailand. Therefore, the purpose of this study was to determine seroprevalence and the risk factors transmitted of Thailand *T. gondii* in dogs and cats in dairy farms in Western part Thailand.

MATERIALS AND METHODS

Animals and blood collections

Blood samples of dogs and cats were collected from 13 positive dairy farms. Dogs and cats from 27 nearby farms were also randomly collected as control in negative herds between June 2007 and May 2008. A total of 114 dogs and 36 cats from 40 dairy herds were examined in this study. Questionnaire for pet owners included information on age, sex, feeding habits (drinking raw milk), pet born in farm, illness history, and owners data (eating raw meat, pregnancy abnormality) and were obtained during farm visits. The blood was centrifuged at 1000 x g for 10 min and the serum separated and stored at -20˚C until serological analysis.

Serological assay

All sera were examined with commercial latex agglutination test (LAT) kits (TOXOCHECK-MT; Eiken Chemical, Tanabe, Japan). This test was evaluated as a screening serologic test for toxoplasmosis in animals (Tsubota et al., 1977a, b). The procedure was described in a previous report (Jittapalapong et al., 2005). Twenty-five microliters of latex agglutination buffer was added to each well of a U-shaped 96 well cluster plate. Then 25 µl of 1:8 diluted sera was mixed with the buffer in the first well. Serial two-fold dilutions were performed in all wells and the final 25 µl was discarded. Then, 25 µl of *T. gondii*-antigen-coated latex beads were added to each well. The plate was shaken gently and incubated at room temperature overnight. The cut-off titer for this test was 1:64 in accordance to the manufacturer's instructions.

Statistical analysis

Descriptive statistics were performed and unconditional associations between disease status and each variable were determined. Eight variables used in pet analysis included sex (male, female), age (≤ 1 year, >1 year), dogs and cats born on the farm, pets illness during year one of the study, pet dying during year one of the study, feces contamination in feed, habit of owners drinking raw milk, and pregnancy abnormality in owners. The variables which *P*-values < 0.10 from univariate analysis were analyzed with multiple logistic regression (Backward stepwise) to identify risk factors associated with *T. gondii* infection at P <0.05 (two-sided), based on the likelihood ratio chi-square test. Goodness-of-fit of the final model was examined to assess how well the model fit the observed data. All analyses were conducted using the statistical software package STATA (version 8.2, Stata Corp., College Station, TX (2003))

RESULTS

A total of 13 toxoplasmosis-positive herds, with 27 toxoplasmosis-negative farms randomly selected as the control were used. The numbers of pets from positive and negative herds are shown in Table 1. Overall seroprevalence of *T. gondii* infections in dogs and cats were 6.1% (7/114) and 8.3% (3/36), respectively. The positives ranged from 1:64 to 1:256 in dogs and from 1:256 to 1:2,048 in cats (Table 2). The seropositive rates of dogs and cats in Kanchanaburi, Nakhon Pathom and Ratchaburi were 14.8% (4/27), 5.1% (2/39), and 4.8% (4/84), respectively. There were two variables significant in unconditional association but not significant in final model. Toxoplasmosis-positive herds with the presence of a pet have a 4.6 times (OR = 4.6, 95%CI: 0.95, 22.5; P = 0.06) higher chance of fecal contamination compared to herds without the presence of pets (Table 3). In toxoplasmosis-positive pet households, one case of pregnancy abnormality was found. However, no documented proof was recorded to show a direct link to the disease. Herds with positive pets, were 6.9 times (OR = 6.9, 95% CI; 1.06-50.8; P = 0.01) more likely to have positive cows (Table 4).

DISCUSSION

The prevalence of dogs and cats in dairy farm was lower

Table 1. Number of pets in positive and negative herds in the case-control study.

Species	Positive herds	Negative herds	Total
Dogs	42	72	114
Cats	11	25	36
Total	53	97	150

Table 2. Antibody titer to *T. gondii* infections of cats and dogs.

Species	No. tested	Frequency of the antibody titer					
		1:64	1:128	1:256	1:512	1:1024	1:1028
Cats	36	0	0	1	1	0	1
Dogs	114	1	1	5	0	0	0

Table 3. Factors related to *T. gondii* infection in pets, OR and P value.

Variable[a]	No. of seronegative	No. of seropositive	OR	95% CI	P value
Sex			0.75	[0.20,2.76]	0.66
Male	74	6			
Female	66	4			
Age			1.88	[0.51,6.98]	0.34
≤ 1 year	78	4			
>1 year	62	6			
Born in farm			0.83	[0.22,3.09]	0.78
Yes	90	6			
No	50	4			
Illness			0.90	[0.22,3.66]	0.89
Yes	45	3			
No	95	7			
Death			0.86	[0.23,3.19]	0.83
Yes	61	4			
No	79	6			
Fecal contamination			4.61	[0.95,22.5]	0.06
Yes	65	8			
No	75	2			
Drinking raw milk			3.73	[0.45,30.4]	0.22
Yes	99	9			
No	41	1			
Pregnancy abnormality			7.67	[0.64,92.7]	0.10
Yes	9	1			
No	138	**2**			

[a]Variable comprises of sex (male, female), age (≤ 1 year, >1 year), pet born on farm or not, illness status, pet died during one year period, feces contamination in feed, owner drinking raw milk, and pregnancy abnormality in owner.

than street dogs. Jittapalapong et al. (2007) reported the prevalence of *T. gondii* in stray dogs in Bangkok was 9.4% (40/427) and *T. gondii* antibodies were detected in 65 (11.0%) of the 592 cats.

In this study, the sex of dogs was not significantly associated with seroprevalence, as has been reported by other researchers (Jittapalapong et al., 2007). No significant differences were observed in the seroprevalence of *T. gondii* in both the sexes of cats as similar to study in Japan (Maruyama et al., 2003).

Table 4. Numbers of positive and negative pets in positive and negative dairy herds.

Species	Positive herds	Negative herds	Total
Positive pet	6	3	9
Negative pet	7	24	31
Total	13	27	40

For the seropositive pets, fecal transmission was the major cause of transmission by 4.6 times more likely to be positive in fecal contamination herds. Cats are the natural reservoir of *T. gondii* and excrete the resistant oocysts to environments. Therefore, cats should be not allow in farm areas and should be kept in the house.

In positive households, one case of pregnancy abnormality may be interpreted as demonstrating the risk as being 7.7 times more likely than of negative households. However, no documented proof was recorded to show a direct link to the disease since the details of pregnancy abnormality were not investigated in this study. Women infected with *T. gondii* for the first time during early pregnancy and immunosuppressed patients are at serious risk for clinical toxoplasmosis (Luft et al., 1984; Chintana et al., 1998). In humans, *T. gondii* is transmitted by several routes such as direct animal contact, eating raw or undercooked meat, and unpasteurised milk containing the parasites in the infective stage (Dubey, 2010). *T. gondii* is not simply another protozoan that ought to be considered in the differential diagnosis of protozoal abortion in cattle. It has great implications concerning public health since the consumption of infected meat or milk can facilitate zoonotic transmission.

This study showed the association between seropositive dogs and cats and positive dairy cows. In herds with positive pets on the farm, the prevalence was 6.9 times more likely to have positive cows in the herd and was highly associated with reproductive problems in dairy herds. Most dairy farms in the Western part Thailand had cats and dogs as pets and no boundary between dairy farm and their houses. Cats are capable of roaming all areas including the food storage and even in stalls. Therefore, the spreading of toxoplasmosis in dairy farms might rely on the number of dogs and cats roaming in the farm. The increasing number of pet will increase the risk of toxoplasmosis among animals in dairy farms.

Conclusions

In conclusions, our results showed that *T. gondii* infection was present in dogs and cats in dairy farms in western province. In herds with positive pets on the farm, the prevalence of having positive cows in the herd was 6.9 times higher. Fecal contamination of feed was found to be a major source of transmission in the study. It is essential to control the number of pets on the farms in order to reduce the transmission of toxoplasmosis in both animals and humans.

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