Tropical Biomedicine 34(2): 419–424 (2017)

Seroprevalence of *Toxoplasma gondii* in free range chickens (*Gallus domesticus*) in Khon Kaen province, Thailand

Saichua, P.[1*], Jumnainsong, A.[2], Tantrawatpan, C.[1], Kiatsopit, N.[3], Kopolrat, K.[3], Suwannatrai, A.[3] and Sithithaworn, P.[3]

[1]Department of Preclinical Science, Faculty of Medicine, Thammasat University, Phahonyothin Road, Pathum Thani, 12120 Thailand

[2]The Centre for Research and Development of Medical Diagnostic Laboratories, Faculty of Associated Medical Science, Khon Kaen University, Mittraphap Road, Khon Kaen, 40002, Thailand

[3]Department of Parasitology, Faculty of Medicine, Khon Kaen University, Mittraphap Road, Khon Kaen, 40002, Thailand

[*]Corresponding author e-mail: psaichua@hotmail.com

Received 31 October 2016; received in revised form 2 February 2017; accepted 4 February 2017

Abstract. Toxoplasmosis is caused by the infective stage of *Toxoplasma gondii* and is often acquired from contaminated food and water. Data on the prevalence of *T. gondii* in free-range chickens (*Gallus domesticus*) in Khon Kaen province, Northeast Thailand, are limited. A total of 257 serum samples were collected and antibodies to *T. gondii* were examined by the latex agglutination test; 26 (10.1%) free-range chickens were positive. By logistic regression, the seroprevalence rate was 3.8 fold higher in the rainy season compared to the dry season (OR=3.81, 95% CI=1.39-10.47, *P*=0.006). The mean rainfall in the seropositive group (3.48 ± 2.05 mm) was significantly higher (*P*=0.028) compared to the seronegative group (2.42 ± 2.35 mm). The point biserial correlation coefficients showed a positive association between seropositivity and mean rainfall (r=0.137, *P*=0.028) but no significant associations for temperature or humidity. In conclusion, the presence of *T. gondii* infection in free-range chickens in Khon Kaen province suggests environmental contamination. The wet season and mean rainfall are significant associations with seroprevalence. The prevention of faecal contamination from cats to the environment is a good strategy to reduce the risk of infection in soil feeding animal such as chicken.

INTRODUCTION

Toxoplasmosis is a worldwide zoonotic disease caused by *Toxoplasma gondii*. It may cause abortion and congenital disease not only in humans but also in intermediate hosts like sheep (Tenter *et al*., 2000, Pappas *et al*., 2009). Toxoplasmosis may be asymptomatic in normal hosts but can cause severe disease in immunodecient hosts (Subauste *et al*., 2011). In Thailand, toxoplasmosis has been detected in pregnant women in several provinces (Wanachiwanawin *et al*., 2001; Dubey 2009; Nissapatorn *et al*., 2011; Sakae *et al*., 2013). The infective oocyst of *T. gondii* is resistant to environmental changes and contaminates soil, fruits, vegetables and water resources. The natural intermediate hosts, rodents or poultry, are the source of the bradyzoite (Dubey 2008). Humans became infected after consuming bradyzoite in meat or oocyst-contaminated drinking water. There is a positive relationship between human seropositivity and the consumption of raw or undercooked meat (Daryani *et al*., 2014; Cong *et al*., 2015; Li *et al*., 2015; Wilking *et al*., 2016; Zhang *et al*., 2016).

Toxoplasma gondii -infected poultry, such as chickens, ducks and pigeons, is consumed widely in many countries (Sreekumar *et al*., 2003; Dubey *et al*., 2008;

Yan *et al*., 2009; Lelu *et al*., 2012; Xu *et al*., 2012; Guo *et al*., 2015), including Thailand (Chumpolbanchorn *et al*., 2009; Unjit 2009). The latex agglutination test (LAT) is a commercial test kit which has a high sensitivity and specificity for detecting anti-Toxoplasma IgG antibodies (Sukthana *et al*., 2001). In Thailand, there are only two seroprevalence studies in free-range chickens (Gallus domesticus) from Chiang Mai and Bangkok (Chumpolbanchorn *et al*., 2009; Unjit 2009). We, therefore, conducted a seroprevalence survey in free-range chickens from Khon Kaen province in northeast Thailand.

MATERIAL AND METHOD

Area of study and sampling

The study was conducted in Khon Kaen province, Northeast Thailand, between May 2014 and June 2015. A total of 257 free-range chickens (*Gallus domesticus*) were randomly selected from households in seven districts. The range of monthly mean rainfall, humidity and temperature are 0–8.6 mm, 58–82% and 22.6–30.7ºC, respectively (data from Thai Meteorological Department). Blood samples were collected by puncture of the wing vein in slaughter houses. Blood samples were allowed to clot at room temperature for 30 minutes after collection, centrifuged and the serum aliquot stored at -20ºC. This study was approved by the Animal Ethics Committee of Faculty of Medicine Thammasat University (No. AE 008/2014).

Latex agglutination test (Toxo-Screen DA, bioMérieux, France)

Serum samples were tested for IgG antibodies using a direct agglutination commercial kit (Toxo-Screen DA®, bioMérieux, France). Briefly, the serum samples were diluted in PBS buffer to which 2-mercaptoethanol was added to denature the IgM. Then the formalin-treated *Toxoplasma* antigen was added to the diluted sample. The results were read after the reaction was left standing for 5 hours at room temperature. A positive reaction between specific IgG antibody in the serum sample and the formalin-treated *Toxoplasma* antigen was indicated by a mat covering. The cut-off point for a positive reaction was a titer at 1:40 (Gebremedhin *et al*., 2014; Gebremedhin *et al*., 2015).

Statistical analysis

The statistical analyses were performed using SPSS for windows (SPSS Inc., Chicago, America). The mean differences between rainfall, humidity or temperature and seropositive and seronegative chickens were compared using the t-test. The Chi squared test was used to determine the association of season (dry and wet) and seroprevalence. Odds ratio (OR) and 95% confidence interval (CI) were calculated. The correlation between rainfall, humidity, temperature and *T. gondii* prevalence were evaluated using point biserial correlation coefficients. All tests were two sided and a P value <0.05 was considered statistically significant.

RESULTS

Overall, *T. gondii* IgG antibodies were detected in 26 of 257 free-range chickens (10.12%); these rates varied from 0 to 50% by location (Table 1). The seroprevalence rates by season were 14.79% (21/142) and 4.35% (5/115) in the wet and dry seasons, respectively, for an OR=3.81 [95% CI=1.39-10.47, P=0.006, (Table 2)]. The mean rainfall was also significantly different (*P*=0.028) between seropositive (3.48 ± 2.05 mm) and seronegative chickens (2.42 ± 2.35 mm.). The point biserial correlation (Table 3) showed that seropositivity was correlated independently with the monthly rainfall but not with either temperature or humidity.

DISCUSSION

This is the first report of the *T. gondii* seroprevalence rates in free-range chickens from Khon Kaen province. The overall rate was high, just over 10%, with widely varying rates by location. The free-rang chicken become infected with *T. gondii* during feeding on the ground (Devada *et al*., 1998)

Table 1. Seroprevalence rates of *T. gondii* infection in free range chickens in Khon Kaen province by sub district using the latex agglutination test

Sub district	District	No. of sample	No. positive	Prevalence (%)
Don Mong	Nong Ruea	55	3	5.45
Phu Wiang	Phu Wiang	8	0	0
Non Phayom	Chonnabot	10	5	50.0
Non Sila	Non Sila	10	0	0
Ban Phai	Ban Phai	49	10	20.41
Ban Fang	Ban Fang	50	5	10.0
Nong bua	Ban Fang	10	0	0
Bueng Niam	Muang	10	1	10.0
Phra Lub	Muang	25	1	4.0
Sila	Muang	30	1	3.33
Total		257	26	10.1

Table 2. Odds ratios for season of free range chicken as risk factors for *T. gondii* in infection in 257 free range chickens in Khon Kaen province

Season	Prevalence	Odd ratio	95% CI [a]	P-value
Dry	4.35 (5/115)	–	–	
Wet	14.79 (21/142)	3.81	1.39-10.47	0.006

[a] CI, confidence interval

Table 3. Point Biserial Correlation Coefficients between IgG seropositivity and rainfall, temperature and humidity

Dependent variable	Independent variable	Point Biserial Correlation Coefficients (r)	P-value
LAT	Rainfall	0.137	0.028
	Temperature	0.114	0.068
	Humidity	0.108	0.085

and should be consider as a source of infection. Previous studies in free-range chickens have reported *T. gondii* seroprevalence rates of 24.3% from Northern Thailand using LAT (Unjit 2009), and 64% from Central Thailand, using indirect fluorescent antibody testing (Chumpolbanchorn *et al.*, 2009). In many surveys of free-range chickens, across the world, seroprevalence rates have varied between 2 and 100% and were especially high in backyard and free range organic chickens (Dubey 2010). However, the seropositive rate in free-range chicken depends on factors such as detection methods, climate and geographic in each study regions. Our 10% seroprevalence rate is similar to Iran and China (Zia-Ali *et al.*, 2007; Yang *et al.*, 2012). The seroprevalence rate was almost four fold of higher in wet season between May to October and was unrelated to temperature and humidity. Similar findings have been reported from Japan, Spain and Taiwan (Yamaoka & Konishi 1993; Gamarra *et al.*, 2008; Tsai *et al.*, 2008) and are consistent with increase survival of oocysts in the soil during

the rainy season. Indeed, Lele *et al.* have shown that oocysts are survive after 100 days in moist soil (43.7%) compared to oocysts in dry soil (7.4%) (Lelu *et al.*, 2012). Soil contamination with oocysts results from cats shedding oocysts in their faeces which can then be further distributed in the environment by the rain. Chickens and other poultry like turkeys become infected when they eat oocyst-contaminated soil which explains why free-range chickens have much higher seropositive rates compared to industrial or caged chickens, supporting the hypothesis that contact with contaminated soil is an important risk factor (Gamarra *et al.*, 2008; Yan *et al.*, 2009; Alkhaled *et al.*, 2012; Yang *et al.*, 2012; Sá *et al.*, 2016).

Dubey (2010) also reported that cats and rats can become infected when they eat leftover meat from infected free-range chickens that have been slaughtered in the home or in unsupervised slaughter houses. Several researchers have demonstrated viable bradyzoites from chicken meat which can infect mice or cats (Sreekumar *et al.*, 2003; Dubey *et al.*, 2005; Zia-Ali *et al.*, 2007). Additionally, *T. gondii* DNA has been detected in free-range chicken meat and it should be concern to protect contaminated meat during food preparing (Chumpolbanchorn *et al.*, 2013; Fernandes *et al.*, 2016). Given the popularity of eating chicken in countries like Thailand, consuming incompletely cooked chicken is an important source of toxoplasma transmission in humans and may be a greater source of toxoplasmosis than beef or pork (Daryani *et al.*, 2014; Cong *et al.*, 2015; Li *et al.*, 2015; Wilking *et al.*, 2016; Zhang *et al.*, 2016).

Therefore, the control strategies are needed to prevent the transmission of *T. gondii* to humans (Tenter *et al.*, 2000). For instance, meat should not be consumed undercooked, vegetables should be washed, cats should be kept away from children's playgrounds, and rigorous hygiene (hand washing) should be emphasized for those who raise chickens at home. Kijlstra (Kijlstra & Jongert 2009) proposed the strategies to prevent *T. gondii* infection on animal farms using a two pronged approach (pre- and post-harvest). In pre-harvest phase, remove cats from farms and vaccinate them. Additionally, the food and bedding of farm animals should be sterile. The rodent population should be controlled as well. In post-harvest phase, the tissue cysts, the bradyzoite, should be destroyed by freezing, heating and inactivating in salt solution. Monitoring the toxoplasmosis seropositivity rate is a good indicator of success of control measures.

Acknowledgements. The authors thank the Department of Parasitology, Faculty of Medicine, Khon Kaen University for providing the laboratory during study time. The authors thank Dr. Yaowalak Sukthana, Faculty of Tropical Medicine, Mahidol University for advice on the manuscript preparation. The authors also thank Dr. Bob Taylor for reviewing the manuscript. This study was supported by the research fund under TU research Scholar Contact Number: GEN 2/ 49/2556.

REFERENCES

Alkhaled, M.J.A., Yakoob, A.Y. & AL-hamadani, A.H.U. (2012). "An investigation of Toxoplasmosis in Free Range chickens, Industrial chickens and Duck in mid Euphrates area of." *AL-Qadisiya Journal of Veterinary Medicine Sciences* **11**(2): 17-24.

Chumpolbanchorn, K., Anankeatikul, P., Ratanasak, W., Wiengcharoen, J., Thompson, R.C. & Sukthana, Y. (2009). "Prevalence of *Toxoplasma gondii* indirect fluorescent antibodies in naturally- and experimentally-infected chickens (Gallus domesticus) in Thailand." *Acta Parasitologica* **54**(3): 194-196.

Chumpolbanchorn, K., Lymbery, A.J., Pallant, L.J., Pan, S., Sukthana, Y. & Thompson, R.C. (2013). "A high prevalence of Toxoplasma in Australian chickens." *Veterinary Parasitology* **196**: 209-211.

Cong, W., Dong, W., Bai, L., Wang, X.Y., Ni, X.T., Qian, A.D. & Zhu, X.Q. (2015). "Seroprevalence and associated risk factors of *Toxoplasma gondii* infection in psychiatric patients: a case-control study

in eastern China." *Epidemiology and Infection* **143**(14): 3103-3109.

Daryani, A., Sarvi, S., Aarabi, M., Mizani, A., Ahmadpour, E., Shokri, A., Rahimi, M.T. & Sharif, M. (2014). "Seroprevalence of *Toxoplasma gondii* in the Iranian general population: a systematic review and meta-analysis." *Acta Tropica* **137**: 185-194.

Devada, K., Anandan, R. & Dubey, J.P. (1998). "Serologic prevalence of *Toxoplasma gondii* in chickens in Madras, India." *Journal of Parasitology* **84**(3): 621-622.

Dubey, J.P. (2008). "The history of *Toxoplasma gondii* – the first 100 years." *The Journal of Eukaryotic Microbiology* **55**(6): 467-475.

Dubey, J.P. (2009). "History of the discovery of the life cycle of *Toxoplasma gondii*." *International Journal for Parasitology* **39**(8): 877-882.

Dubey, J.P. (2010). "*Toxoplasma gondii* infections in chickens (Gallus domesticus): prevalence, clinical disease, diagnosis and public health significance." *Zoonoses Public Health* **57**(1): 60-73.

Dubey, J.P., Edelhofer, R., Marcet, P., Vianna, M.C., Kwok, O.C. & Lehmann, T. (2005). "Genetic and biologic characteristics of *Toxoplasma gondii* infections in free-range chickens from Austria." *Veterinary Parasitology* **133**(4): 299-306.

Dubey, J.P., Huong, L.T., Lawson, B.W., Subekti, D.T., Tassi, P., Cabaj, W., Sundar, N., Velmurugan, G.V., Kwok, O.C. & Su, C. (2008). "Seroprevalence and isolation of *Toxoplasma gondii* from free-range chickens in Ghana, Indonesia, Italy, Poland, and Vietnam." *The Journal of Parasitology* **94**(1): 68-71.

Gamarra, J.A., Cabezon, O., Pabon, M., Arnal, M.C., Luco, D.F., Dubey, J.P., Gortazar, C. & Almeria, S. (2008). "Prevalence of antibodies against *Toxoplasma gondii* in roe deer from Spain." *Veterinary Parasitology* **153**(1-2): 152-156.

Gebremedhin, E.Z., Abdurahaman, M., Hadush, T. & Tessema, T.S. (2014). "Seroprevalence and risk factors of *Toxoplasma gondii* infection in sheep and goats slaughtered for human consumption in Central Ethiopia." *BMC Research Notes* **7**: 696.

Gebremedhin, E.Z., Tesfamaryam, G., Yunus, H.A., Duguma, R., G. Tilahun, D.I.M.V. & Vitale, M. (2015). "Seroepidemiology of *Toxoplasma gondii* infection in free-range chickens (Gallus domesticus) of Central Ethiopia." *Epidemiology and Infection* **143**(3): 608-617.

Guo, M., Dubey, J.P., Hill, D., Buchanan, R.L., Gamble, H.R., Jones, J.L. & Pradhan, A.K. (2015). "Prevalence and risk factors for *Toxoplasma gondii* infection in meat animals and meat products destined for human consumption." *Journal of Food Protection* **78**(2): 457-476.

Kijlstra, A. & Jongert, E. (2009). "Toxoplasma-safe meat: close to reality?" *Trends in Parasitology* **25**(1): 18-22.

Lelu, M., Villena, I., Darde, M.L., Aubert, D., Geers, R., Dupuis, E., Marnef, F., Poulle, M.L., Gotteland, C., Dumetre, A. & Gilot-Fromont, E. (2012). "Quantitative estimation of the viability of *Toxoplasma gondii* oocysts in soil." *Applied and Environmental Microbiology* **78**(15): 5127-5132.

Li, H.L., Dong, L., Li, Q., Zhang, L., Chen, J., Zou, F.C. & Zhu, X.Q. (2015). "Seroepidemiology of *Toxoplasma gondii* infection in Bai and Han ethnic groups in southwestern China." *Epidemiology and Infection* **143**(4): 881-886.

Nissapatorn, V., Suwanrath, C., Sawangjaroen, N., Ling, L.Y. & Chandeying, V. (2011). "Toxoplasmosis-serological evidence and associated risk factors among pregnant women in southern Thailand." *The American Journal of Tropical Medicine and Hygiene* **85**(2): 243-247.

Pappas, G., Roussos, N. & Falagas, M.E. (2009). "Toxoplasmosis snapshots: global status of *Toxoplasma gondii* seroprevalence and implications for pregnancy and congenital toxoplasmosis." *The International Journal for Parasitology* **39**(12): 1385-1394.

Sá, S.G., Lima, D.C., Silva, L.T., Pinheiro, J.W., Dubey, J.P., Silva, J.C. & Mota, R.A. (2016). "Seroprevalence of *Toxoplasma gondii* among turkeys on family farms in the

state of Northeastern Brazil." *Acta Parasitologica* **61**(2): 401-405.

Sakae, C., Natphopsuk, S., Settheetham-Ishida, W. & Ishida, T. (2013). "Low Prevalence of *Toxoplasma gondii* Infection Among Women in Northeastern Thailand." *The Journal of Parasitology* **99**(1): 172-173.

Sreekumar, C., Graham, D.H., Dahl, E., Lehmann, T., Raman, M., Bhalerao, D.P., Vianna, M.C. & Dubey, J.P. (2003). "Genotyping of *Toxoplasma gondii* isolates from chickens from India." *Veterinary Parasitology* **118**(3-4): 187-194.

Subauste, C.S., Ajzenberg, D. & Kijlstra, A. (2011). "Review of the series "Disease of the year 2011: toxoplasmosis" pathophysiology of toxoplasmosis." *Ocular Immunology and Inflammation* **19**(5): 297-306.

Sukthana, Y., Chintana, T., Supatanapong, W., Siripan, C., Lekkla, A. & Cheabchalrad, R. (2001). "Predictive value of latex agglutination test in serological screening for *Toxoplasma gondii*." *The Southeast Asian Journal of Tropical Medicine and Public Health* **32**(2): 314-318.

Tenter, A.M., Heckeroth, A.R. & Weiss, L.M. (2000). "*Toxoplasma gondii*: from animals to humans." *The International Journal for Parasitology* **30**(12-13): 1217-1258.

Tsai, Y.J., Chung, W.C., Fei, A.C., Hong, C.L., Tsai, Y.Y., Peng, S. & Wu, Y.L. (2008). "Prevalence of *Toxoplasma gondii* antibodies in stray dogs in Taipei, Taiwan." *The Journal of Parasitology* **94**(6): 1437.

Unjit, K. (2009). Prevalence of *Toxoplasma gondii* Infection in Free Range Chicken in Chiang Mai, Thailand. Master of Veterinary Public Health, Chiang Mai University.

Wanachiwanawin, D., Sutthent, R., Chokephaibulkit, K., Mahakittikun, V., Ongrotchanakun, J. & Monkong, N. (2001). "*Toxoplasma gondii* antibodies in HIV and non-HIV infected Thai pregnant women." *Asian Pacific Journal of Allergy and Immunology* **19**(4): 291-293.

Wilking, H., Thamm, M., Stark, K., Aebischer, T. & Seeber, F. (2016). "Prevalence, incidence estimations, and risk factors of *Toxoplasma gondii* infection in Germany: a representative, cross-sectional, serological study." *Scientific Reports* **6**: 22551.

Xu, P., Song, X., Wang, W., Wang, F., Cao, L. & Liu, Q. (2012). "Seroprevalence of *Toxoplasma gondii* infection in chickens in Jinzhou, northeastern China." *The Journal of Parasitology* **98**(6): 1300-1301.

Yamaoka, M. & Konishi, E. (1993). "Prevalence of antibody to *Toxoplasma gondii* among inhabitants under different geographical and climatic conditions in Hyogo Prefecture, Japan." *Japanese Journal of Medical Science and Biology* **46**(3): 121-129.

Yan, C., Yue, C.L., Yuan, Z.G., He, Y., Yin, C.C., Lin, R.Q., Dubey, J.P. & Zhu, X.Q. (2009). "*Toxoplasma gondii* infection in domestic ducks, free-range and caged chickens in southern China." *Veterinary Parasitology* **165**(3-4): 337-340.

Yang, N., Mu, M.Y., Li, H.K., Long, M. & He, J.B. (2012). "Seroprevalence of *Toxoplasma gondii* infection in slaughtered chickens, ducks, and geese in Shenyang, northeastern China." *Parasites & Vectors* **5**: 237.

Zhang, X.X., Zhao, Q., Shi, C.W., Yang, W.T., Jiang, Y.L., Wei, Z.T., Wang, C.F. & Yang, G.L. (2016). "Seroprevalence and associated risk factors of *Toxoplasma gondii* infection in the Korean, Manchu, Mongol and Han ethnic groups in eastern and northeastern China." *Epidemiology and Infection* **144**(9): 2018-2024.

Zia-Ali, N., Fazaeli, A., Khoramizadeh, M., Ajzenberg, D., Darde, M. & Keshavarz-Valian, H. (2007). "Isolation and molecular characterization of *Toxoplasma gondii* strains from different hosts in Iran." *Parasitology Research* **101**(1): 111-115.